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With sixteen Plates and four hundred and ninety-four Figures in the Text

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CONTENTS.

No. CXCIII, January, 1935.

	PAGE
COLSON, BARBARA.—The Cytology of the Mushroom <i>Psalliota campestris</i> Quel. With forty-nine Figures in the Text	I
NIRIMARGI, N. M.—Studies in the Genera <i>Cytosporina</i> , <i>Phomopsis</i> and <i>Diaporthe</i> . VII. Chemical Factors Influencing Sporing Characters. With eight Figures in the Text	19
WEBB, PHILIP C. R.—The Cytology and Life-history of <i>Sorosphaera Veronicæ</i> . With four Figures in the Text	41
DASTUR, R. H., and DESAI, R. M.—The Carbon Dioxide Carbohydrate Ratio in the Aerobic and the Anaerobic Respiration of Rice. With four Figures in the Text	53
PADY, S. M.—A Cytological Study of the Development and Germination of the Teliospores of <i>Hyalospora aspidiotus</i> (Pk.) Magn. With fifty-four Figures in the Text	71
ASHWORTH, DOROTHY.—The Receptive Hyphae of the Rust Fungi. With five Figures in the Text	95
ISAAC, W. E.—A Preliminary Study of the Water Loss of <i>Laminaria digitata</i> During Intertidal Exposure. With one Graph in the Text	109
HUSKINS, C. LEONARD, and SMITH, STANLEY G.—Meiotic Chromosome Structure in <i>Trillium erectum</i> L. With Plates I to III and eight Figures in the Text	119
SASS, J. E.—Cytological Aspects of Physiological Sterility in <i>Coprinus sterquilinus</i> Fr. With Plate IV	151
BENSON, M.—The Fructification, <i>Calathiops Bernhardtii</i> , n. sp. With Plate V and three Figures in the Text	155
PRIESTLEY, J. H., SCOTT, L. I., and GILLETT, E. C.—The Development of the Shoot in <i>Alstroemeria</i> and the Unit of Shoot Growth in Monocotyledons. With eleven Figures in the Text	161

No. CXCIV, April, 1935.

DICKSON, HUGH.—Studies in <i>Coprinus sphaerosporus</i> . II. The Inheritance of Various Morphological and Physiological Characters. With Plate VI and fourteen Figures in the Text	181
NAYAL, A. A.—Two New Members of the Chaetophorales from Egypt. With Plate VII and five Figures in the Text	205
HAINES, F. M.—Transpiration and Pressure Deficit. I. Apparatus and Preliminary Experiments. With three Figures in the Text	213
HOLDEN, H. S.—On the Structure and Probable Affinities of Some Leaflets from Autun. With Plate VIII and two Figures in the Text	239
LOOMIS, W. E.—Translocation and Growth Balance in Woody Plants. With twelve Figures in the Text	247
DASTUR, R. H., and GUNJIKAR, L. K.—Energy Absorption by Leaves in Normal and Plane Polarized Light. With one Figure in the Text	273
PARKIN, JOHN.—The Structure of the Starch Layer in the Glossy Petal of <i>Ranunculus</i> . II. The British Species Examined. With nine Figures in the Text	283
SINGH, B. N., and LAL, K. N.—Investigation of the Effect of Age on Assimilation of Leaves. With six Figures in the Text	291
ASHBY, ERIC, and OXLEY, T. A.—The Interaction of Factors in the Growth of <i>Lemna</i> . VI. An Analysis of the Influence of Light Intensity and Temperature on the Assimilation Rate and the Rate of Frond Multiplication. With fifteen Figures in the Text	309

	PAGE
ARBER, AGNES.—The 'Needles' of Asparagus, with Special Reference to A. Sprengeri Reg. With three Figures in the Text	337
BAPTISTE, E. C. D.—The Effect of Some Cations on the Permeability of Cells to Water. With thirteen Figures in the Text	345
HAINES, F. M.—Observations on the Occurrence of Air in Conducting Tracts	367
NEWTON, R. G.—An Improved Electrical Conductivity Method for the Estimation of Carbon Dioxide and other Reactive Gases. With eight Figures in the Text	381
NOTES.	
JANE, FRANK W.—A 'Back' for Use when Honing Microtome Knives. With one Figure in the Text	390
HOLDEN, H. S.—A New Type of Culture Vessel. With one Figure in the Text	401

No. CXCIV, July, 1935.

LAMB, I. M.—The Initiation of the Dikaryophase in Puccinia phragmitis (Schum.) Körn. With Plates IX and X	403
DREW, KATHLEEN M.—The Life-history of Rhodochorton violaceum (Kütz.) comb. nov. (Chantrya violacea Kütz.). With nineteen Figures in the Text	439
SMITH, G. E.—On the Orientation of Stomata. With fifteen Figures in the Text	451
COLLINS, E. J.—The Problem of Immunity to Wart Disease [Synchytrium endobioticum, (Schilb.) Perc.] in the Potato	479
WICKS, L. M.—The Anatomy of Amaryllidaceous Leaves. I. Stomatal Distribution in Haemanthus and Brunsvigia. With Plate XI and eleven Figures in the Text	493
EWART, A. J.—Disarticulation of the Branches in Eucalyptus. With Plate XII and one Figure in the Text	507
HOLLOWAY, J. E.—The Gametophyte of Phylloglossum Drummondii. With six Figures in the Text	513
HAINES, F. M.—Transpiration and Pressure Deficit II. With seven figures in the Text	521
LURGESS, A. H.—The Effect of the Rate of Flow of Air upon Assimilation and of Fluids upon Other Natural Processes. With one Figure in the Text	567
DARLINGTON, C. D.—The Old Terminology and the New Analysis of Chromosome Behaviour. With one Figure in the Text	579
GRAHAM, ROY.—An Anatomical Study of the Leaves of the Carboniferous Arborescent Lycopods. With fifty-three Figures in the Text	587
HAAS, P., HILL, T. G., and KARSTENS, W. K. H.—The Metabolism of Calcareous Algae. II. The Seasonal Variation in Certain Metabolic Products of Corallina squamata Ellis. With six Figures in the Text	609

NOTE.

ROSENBERG, M.—On the Germination of Lemanea torulosa in Culture. With two Figures in the Text	621
---	-----

No. CXCVI, October, 1935.

HATCH, WINSLOW R.—Gametogenesis in Allomyces arbuscula. With thirty-three Figures in the Text	623
BLOCH, ROBERT.—Wound Healing in Tradescantia fluminensis Vell. With eleven Figures in the Text	651
ANDERSON, EDGAR, and DE WINTON, DOROTHEA.—The Genetics of Primula sinensis. IV. Indications as to the Ontogenetic Relationship of Leaf and Inflorescence. With Plates XIII and XIV and ten Figures in the Text	671
SUTTON, E.—Half-disjunction in an Association of Four Chromosomes in Pisum sativum. With seven Figures in the Text	689
JONES, S. G.—The Structure of Lophodermium pinastri (Schrad.), Chev. With twenty Figures in the Text	699
HAVAS, LÁSZLÓ, and CALDWELL, JOHN.—Some Experiments on the Effects of Animal Hormones on Plants. With Plate XV and one Figure in the Text	729

	PAGE
BLACKMAN, G. E.—A Study by Statistical Methods of the Distribution of Species in Grass-land Associations. With an Appendix by M. S. BARTLETT. With thirteen Figures in the Text	749
ASHBY, ERIC.—The Quantitative Analysis of Vegetation. With an Appendix by W. L. STEVENS. With nine Figures in the Text	779
BOLAS, B. D., and SELMAN, I. W.—An Inexpensive Recording Porometer. With six Figures in the Text	803
DASTUR, R. H., and MEHTA, R. J.—The Study of the Effect of Blue-violet Rays on Photosynthesis	809
OLIVER, F. W.—Dukinfield Henry Scott, 1854-1934. With a Portrait	823

NOTES.

JOSHI, A. C., and VENKATESWARLU, J.—A Case of Reversed Polarity in the Embryo-sac. With three Figures in the Text	841
BOWEN, ESTHER J.—A Note on the Conduction of Water in <i>Fimbriaria bleumeana</i> . With six Figures in the Text	844
SAUNDERS, EDITH R.—On the Gynaeceum of <i>Filipendula Ulmaria</i> Maxim. and <i>Filipendula hexapetala</i> Gillib. : A Correction. With eleven Figures in the Text	848
DOBBS, C. G.—Two Types of Modified Petri Dish. With two Figures in the Text	852
HENDERSON, F. Y.—Timber and Attack by <i>Lyctus</i> Beetle	854

	PAGE
ROSENBERG, M.—On the Germination of <i>Lemanea torulosa</i> in Culture. With two Figures in the Text	621
SASS, J. E.—Cytological Aspects of Physiological Sterility in <i>Coprinus sterquilinus</i> Fr. With Plate IV	151
SAUNDERS, E. R.—On the Gynaceum of <i>Filipendula Ulmaria</i> Maxim. and <i>Filipendula hexapetala</i> Gillib. : A Correction. With eleven Figures in the Text	848
SCOTT, L. I., see PRIESTLEY, J. H.	
SELMAN, I. W., see BOLAS, B. D.	
SINGH, B. N., and LAL, K. N.—Investigation of the Effect of Age on Assimilation of Leaves. With six Figures in the Text	291
SMITH, G. E.—On the Orientation of Stomata. With fifteen Figures in the Text . .	451
SMITH, S. G., see HUSKINS, C. L.	
STEVENS, W. L., see ASHEY, E.	
SUTTON, E.—Half-disjunction in an Association of Four Chromosomes in <i>Pisum sativum</i> . With seven Figures in the Text	689
VENKATESWARLU, J., see JOSHI, A. C.	
WEBB, P. C. R.—The Cytology and Life-history of <i>Sorosphaera Veronicae</i> . With four Figures in the Text	41
WICKS, L. M.—The Anatomy of Amaryllidaceous Leaves. I. Stomatal Distribution in <i>Haemanthus</i> and <i>Brunsvigia</i> . With Plate XI and eleven Figures in the Text .	493

B. ILLUSTRATIONS.

- a. PLATES. I-III. *Trillium* (HUSKINS and SMITH).
 [IV. Sterility in *Coprinus* (SASS).
 V. *Calathiops* (BENSON).
 VI. *Coprinus sphaerosporus* (DICKSON).
 VII. *Chaetophorales* (NAVAL).
 [VIII. Leaflets from *Autun* (HOLDEN).
 IX-X. *Dikaryophase* in *Puccinia phragmitis* (LAMB).
 XI. *Haemanthus* and *Brunsvigia* (WICKS).
 XII. *Eucalyptus macrorrhyncha* (EWART).
 XIII-XIV. *Primula sinensis* (ANDERSON and DE WINTON).
 XV. Effect of Animal Hormones on Plants (HAVAS and CALDWELL).
 Portrait of Dukinfield Henry Scott (OLIVER).
- b. FIGURES.
- 1-8. 1. Portion of cell of mycelial strand connecting fructification to subterranean mycelium. 2. Part of cell of undifferentiated fructification. 3. Parts of two cells of stipe of young fructification. 4. Binucleate cells of sub-hymenium and basidia. 5. Cell of pileus of mature fructification. 6. Multinucleate cell of stipe. 7. Binucleate cell of trama. 8. Four-nucleate cell of trama (COLSON) 5
- 9-17. 9. Young binucleate basidium. 10. Nuclear fusion in basidium. 11. Definitive nucleus of basidium. 12. Late diakinesis. 13. a. Prophase of first division in basidium; b. Early diakinesis; c. Metaphase of first division; d. Early anaphase with seventeen chromosomes. 14. Early anaphase with eighteen chromosomes. 15. Binucleate basidium after first division. 16. Metaphase of second division. 17. Late anaphase of second division (COLSON) 7
- 18-23. Two-spored form. 18. Four-nucleate basidium. 19. Young sterigmata. 20. Young spores. 21. Thin-walled spores, nuclei in base of basidium. 22. Thick-walled spores, nuclei bearing sterigmata. 23. Three nuclei in basidium, one in sterigma (COLSON) 9
- 24-30. Two-spored form. 24. Nucleus in each sterigma; two nuclei still in basidium preparing to enter sterigmata. 25-6. Nucleus in each spore and each sterigma.

FIGURES.

PAGE

	27. Spores artificially detached; whole nucleus in each spore, part of a second nucleus in spore and part in sterigma. 28. Binucleate spores. 29. Four-nucleate spores. 30. Eight-nucleate spore (COLSON)	11
31-7.	Four-spored form. 31. Late diakinesis with nine gemini. 32. Metaphase of first division. 33. Four-nucleate basidium. 34. Young sterigmata. 35. Young spores. 36. Thin-walled spores, nuclei low in obliquely cut basidium. 37. Thick-walled spores (COLSON)	12
38-42.	Four-spored form. 38. Nuclei moving up to the sterigmata. 39. Cut basidium with two of four spores and sterigmata, each sterigma containing nucleus. 40. Uninucleate spores, basidium empty. 41. <i>a</i> and <i>b</i> , two views of same basidium, broken nuclei, part in spore and part in sterigma, nuclei in spores. 42. Binucleate spores (COLSON)	13
43-9.	Mycelium from spores and tissue cultures. 43. Six-nucleate cell from spore mycelium. 44. Cell with eleven nuclei from tissue culture mycelium. 45. Portion of same cell. 46. Branching of mycelium from spore. 47. Branching of mycelium of tissue culture. 48. Mycelium from spore with granule at septum. 49. Mycelium from tissue culture showing granule (COLSON)	14
1.	Illustrates variation in 'A' spores conditioned by asparagin. <i>Phomopsis coneglanensis</i> , <i>Cytosporina ludibunda</i> , CA ₃₋₁ (NITIMARGI)	25
2.	Illustrates the effect of high concentration of glucose on the spores of <i>P. citri</i> , B 10 (NITIMARGI)	26
3.	Illustrates variation in sporing characters conditioned by sugar, <i>P. coneglanensis</i> , <i>C. ludibunda</i> , CA ₃₋₁ (NITIMARGI)	27
4.	Illustrates variation in sporing characters conditioned by glucose. <i>P. citri</i> , J1 (NITIMARGI)	29
5.	Illustrates variation in sporing characters conditioned by glucose and asparagin. <i>C. ludibunda</i> , CA ₃ (NITIMARGI)	30
6.	Graph showing percentage number of 'B' spores in relation to concentration of glucose for four concentrations of asparagin. <i>C. ludibunda</i> , CA ₃ (NITIMARGI)	32
7.	Graph showing length of 'B' spores in relation to concentration of glucose for four concentrations of asparagin. <i>C. ludibunda</i> , CA ₃ (NITIMARGI)	33
8.	Illustrates variation in sporing characters conditioned by glucose and asparagin. <i>P. coneglanensis</i> (NITIMARGI)	35
1.	The somatic mitosis (WEBB)	43
2.	<i>a-g</i> , heterotype division; <i>r-z</i> , homotype division (WEBB)	46
3-4.	3. Number of nuclei in plasmodia at diakinesis. 4. Number of nuclei in plasmodia at metaphase (WEBB)	47
1.	Graphs showing the rates of aerobic and anaerobic respiration of the seeds of rice germinated in the dark (DASTUR and DESAI)	55
2.	Graphs showing the rates of aerobic and anaerobic respiration in seeds germinated in the dark (DASTUR and DESAI)	56
3.	Graphs showing the rates of aerobic and anaerobic respiration in seeds of rice germinated in light (DASTUR and DESAI)	57
4.	Diagram of the apparatus used for measuring anaerobic respiration in longitudinal section (DASTUR and DESAI)	58
1-5.	1. Mycelium in mesophyll of leaf. 2. Young primordial cell <i>b</i> formed by enlargement of the tip of the hypha <i>a</i> . 3. Group of developing primordial cells in various stages. 4. A single mature primordial cell. 5. Teliospore initials <i>a</i> , <i>b</i> , <i>c</i> , and <i>d</i> in the epidermal cell (PADY)	75
6-22.	6. A young teliospore with empty primordial cell <i>a</i> . 7. Two-celled stage. 8. Three cells of a later stage. 9. A two-celled teliospore. 10, 11. Fusion of the two nuclei. 12. Mature fusion nucleus in resting condition. 13. Early prophase. 14. Later stage. 15. Surface view of three cells showing stages in spireme formation. 16. Synaptic knot. 17-18. Later stage of spireme. 19. Fragmentation of the spireme. 20. Paired chromomeres in the fragmented spireme. 21, 22. Button-like body on outer wall of the teliospore (PADY)	79

FIGURES.

PAGE

	inflorescences and the basal needles; 6-8 show the further history of the shoot; 8 passes through the apical region (ARBER)	340
3.	<i>Asparagus Sprengeri</i> Reg. A and B, sections from transverse series from below upwards through other buds from the same lateral shoot as that shown in Fig. 2. A 1-A 6, a shoot with only one basal lateral shoot of the third order, which is enclosed in the margin of the prophyll of the shoot of the second order. B, a lateral bud in which the right-hand basal shoot bore an inflorescence, but no needle. C, transverse section of a shoot passing through the base of a needle trio, somewhat older than those shown in Figs. 2, 6 and 7 (ARBER)	342
1.	Uptake of water after varying periods of drying of discs (BAPTISTE)	348
2.	Uptake after treatment with KCl, CaCl ₂ and control. Potato (BAPTISTE)	350
3.	Uptake after treatment with KCl, NaCl (two experiments), MgCl ₂ (two experiments) and two controls. Potato (BAPTISTE)	350
4.	Uptake after treatment with MgCl ₂ (two experiments) and controls. Potato (BAPTISTE)	350
5.	Uptake after treatment with NH ₄ Cl and control. Potato (BAPTISTE)	350
6.	Uptake after treatment with NH ₄ Cl, MgCl ₂ and control. Potato (BAPTISTE)	351
7.	Uptake after treatment with NH ₄ Cl, CaCl ₂ and control. Potato (BAPTISTE)	351
8.	Uptake after treatment with KCl, MgCl ₂ (two experiments) and controls. Carrot (BAPTISTE)	354
9.	Uptake after treatment with NaCl, CaCl ₂ and control. Carrot (BAPTISTE)	355
10.	Water-loss in hypertonic sugar solutions (BAPTISTE)	356
11.	Relation between reduction in percentage water content for potato discs immersed in sugar solutions of known osmotic pressure (BAPTISTE)	357
12.	Relation between percentage change in water content and osmotic pressure of sugar solutions (BAPTISTE)	359
13.	Curves for water uptake in hypertonic sugar solutions by discs previously dried for 15 minutes over CaCl ₂ (BAPTISTE)	360
1.	Circuit layout (NEWTON)	386
2.	Conductivity cell (NEWTON)	387
3.	Calibration of fifth experimental absorber (NEWTON)	388
4.	Calibration of ninth experimental absorber (NEWTON)	390
5.	Calibration of tenth experimental absorber (NEWTON)	391
6.	Respiration of two <i>pelargonium</i> leaves (NEWTON)	394
7.	Respiration rate of four samples of <i>Lemna minor</i> grown at 1,600 foot candles (NEWTON)	396
8.	Estimation of hydrogen sulphide (NEWTON)	396
1.	Design for 'back' for use when honing plane-faced microtome knives (JANE)	400
1.	New type of culture vessel (HOLDEN)	401
1-10.	1. Small part of plant, showing habit of growth and branchlets bearing monosporangia. 2. Cell of main filament with very lobed plastid and prominent nucleolus. 3. Similar cell with dense parietal plastid. 4. Cell with ribbon-shaped plastids, spirally arranged. 5. Various stages of germination of spore on <i>Lemanea fluviatilis</i> . 6. Filament with branchlets bearing both monosporangia and tetrasporangia. 7. Developing tetrasporangium containing two nuclei. 8. Four-nucleate tetrasporangium, showing the beginning of the transverse division of the protoplast. 9. Slightly older tetrasporangium, the second protoplasmic divisions having begun. 10. Antheridial branchlet with young, mature, and empty antheridia (DREW)	442
11-17.	11. Apical portion of male filament. 12. Filament bearing both antheridia and monosporangia. 13. Filament with carpogonia and also monosporangia. 14. Mature sessile carpogonium with two spermatia adhering to trichogyne. 15. Fertilized carpogonium. 16. Two gonimoblastic filaments developing at apical end of basal part of carpogonium. 17. Later stage in the development of the cystocarp, showing three gonimoblastic filaments (DREW)	444

RES.	PAGE
-8. 6. Nitrogen of bark of base segment. 7. Nitrogen of bark of middle segment. 8. Nitrogen of bark of upper segment (LOOMIS)	259
9. The effect of ringing upon the diameter growth of apple. 1929 experiments (LOOMIS)	261
10. The effect of ringing upon the diameter growth of large apple and box elder. 1932 experiments (LOOMIS)	263
11. The negative correlation between sprout and diameter growth (LOOMIS)	264
12. The relation of soluble nitrogen to sprout and cambial growth (LOOMIS)	267
1. A. A diagrammatic sketch of the apparatus for obtaining normal and plane polar- ized beams of light of equal intensities. B. Sketch of the face view of the leaf attached to the microthermopile (DASTUR and GUNJIKAR)	275
1-9. 1. Transverse and Fig. 2, longitudinal section of the petal of <i>Ranunculus Ficaria</i> L. 3-9. Longitudinal sections of various petals showing only upper epidermis and starch layer. 3. <i>R. bulbosus</i> L. 4. <i>R. repens</i> L. 5. <i>R. sardous</i> Cr. 6. <i>R. parviflorus</i> L. 7. <i>R. auricomus</i> L. 8. <i>R. Flammula</i> L. 9. <i>R. scelerat-</i> <i>us</i> L. (PARKIN)	285
1-3. Effect of age and developmental stage of leaves on photosynthesis. 1. Wheat. 2. Linseed. 3. Sugar-cane (SINGH and LAL)	296
4. Water content of leaves of wheat (P_4) and linseed (1150 C) (SINGH and LAL)	299
5. Inorganic constituents, wheat (P_4) (SINGH and LAL)	300
6. Inorganic constituents, linseed (1150 C) (SINGH and LAL)	301
1. Growth chamber used for cultures of <i>Lemna</i> described in this paper (ASHBY and OXLEY)	312
2. Diagram of photo-electric apparatus for the measurement of frond area (ASHBY and OXLEY)	314
3. Calibration graph for the apparatus for measurement of frond area (ASHBY and OXLEY)	315
4. Logarithms of frond number plotted against time in days (ASHBY and OXLEY)	317
5. Experiment at 35° C. logarithms of frond number plotted against time in days (ASHBY and OXLEY)	325
6. Relative multiplication rates plotted against temperature (ASHBY and OXLEY)	326
7. Relative multiplication rates plotted against light intensity (ASHBY and OXLEY)	327
8. Mean net assimilation rates in milligrams of CO_2 per square decimetre per hour plotted against temperature (ASHBY and OXLEY)	328
9. Mean net assimilation rates in milligrams of CO_2 per square decimetre per hour plotted against light intensity (ASHBY and OXLEY)	330
10. Drawing of a three-dimensional model representing the interaction of light and temperature on relative multiplication rate (ASHBY and OXLEY)	331
11. Drawing of a three-dimensional model representing the interaction of light and temperature on mean net assimilation rate (ASHBY and OXLEY)	331
12. Relative multiplication rate plotted against assimilation rate at different tempera- tures (ASHBY and OXLEY)	332
13. Logarithms of values of ($R-r$) for different temperatures plotted against correspon- ding assimilation rates (ASHBY and OXLEY)	333
14. Mean dry weight per frond plotted against light intensity (ASHBY and OXLEY)	333
15. Mean dry weight per frond plotted against temperature (ASHBY and OXLEY)	334
1. <i>Asparagus Sprengeri</i> Reg., from plants grown under glass, Cambridge Botanic Garden, October, 1934. A, vegetative shoot. B 1, fertile shoot. B 2, part of an infructescence axis with two berries to show the articulation of the pedicel; below the berries is a shrivelled pedicel in which detachment has occurred at the plane of the articulation (ARBER)	339
2. <i>Asparagus Sprengeri</i> Reg. 1-8, transverse sections from a series from below upwards through a young lateral shoot, collected on May 4, 1934, from a plant grown under glass, Cambridge Botanic Garden. In 1, the axillant leaf is not yet completely free from the axis; 2-5 show the origin of the rudimentary	

FIGURES.

PAGE

- 23-37. 23. Germination. 24. Papilla in contact with host cell-wall. 25-8. Passage of contents into young promycelium. 29. Nucleus passing in promycelium. 30. Late prophase. 31. Division figure showing rod-shaped spindle and chromosomes. 32. Chromosomes moving on to spindle. 33. Spindle fibres moving apart. 34-5. Anaphase. 36. Slightly later anaphase. 37. Telophase (PADY) 81
- 38-54. 38. Later telophase. 39-41. Stages in second division. 42. Telophase. 43. Daughter nuclei of second division. 44. Typical four-celled promycelium. 45. Beginning of sterigmata. 46. Young sterigma and young basidiospore. 47-50. Passage of nucleus through sterigmata. 51. Mature basidiospore. 52. Basidiospore with two nuclei. 53, 54. Button-like body on basidiospore wall (LADY) 85
- 1 a-c. *Melampsorium betulinum*. a. Uninucleate hyphae pushing up between the guard cells of a stoma of *Larix europea*, eleven days after infection. b. Inter-cellular emergent hyphae. c. Massing of hyphae below stoma. Twelve days after infection. d. Several hyphae in sub-stomatal space. e. Sub-stomatal space filled with hyphae (ASHWORTH) 100
- 2 a-d. a. *Melampsora larici-populina*. Hyphae in stoma. b. *M. larici-caprearum*. Hypha pushing between the guard cells of a stoma. c. *M. larici-populina*. Branched emergent hypha. d. *Endophyllum sempervivi*. Hyphae emerging between the guard cells of a stoma (ASHWORTH) 102
- 3 a-c. a. *Coleosporium tussilaginis*. Hyphae in the youngest part of the aecidium grouping beneath stomata. b. *C. tussilaginis*. Hyphae at the periphery of the spermogonium pushing up into stomata. c. *Endophyllum sempervivi*. The mycelium in the sub-epidermal region of the leaf (ASHWORTH) 103
- 4 a-c. a. *Phragmidium violaceum*. Inter-cellular hypha at the upper epidermis. b. *P. violaceum*. Inter-cellular hypha at the lower epidermis. c. Hyphae of *P. violaceum* in the region of an aecidium pushing through the lower epidermis (ASHWORTH) 105
- 5 a-e. a. *Puccinia malvacearum*. Hypha crushed between guard cells at the upper epidermis. b. *P. malvacearum*. Emergent hypha. Upper epidermis. c. *Melampsorium betulinum*. Teleutosorus. Teleutospore crushed between the guard cells of the stoma. d. *Puccinia malvacearum*. Hypha emerging through stoma at lower epidermis. e. *Phragmidium violaceum*. Stomatal hypha at the lower epidermis of the uredosorus (ASHWORTH) 107
1. Graph showing variation in the relative humidity of the air near low-water mark (ISAAC). 111
1. Haploid chromosome complement at metaphase of first pollen-grain division (HUSKINS and SMITH). 121
2. a. Late diplotene; b. early diakinesis; c. mid diakinesis; d. late diakinesis; e. metaphase (HUSKINS and SMITH) 121
- 3 a-f. Pachytene chromosomes of the nucleus illustrated in Plate I, Fig. 3, here drawn separately and more diagrammatically (HUSKINS and SMITH) 123
- 4-6. Anaphase configurations of chromosome A illustrating changes in direction of coiling (HUSKINS and SMITH) 130
7. Diagrams illustrating possible interpretations of configurations 4a and b and 5a and b (HUSKINS and SMITH)
- 8 a-d. 'Bridge' chromatid arising through crossing-over in a heterozygous inverted segment in chromosome D (HUSKINS and SMITH)
1. A drawing from the stone of the whole specimen of *Calathiops Bernhardti*, n. sp. (BENSON)
2. An enlarged drawing from the stone at C' in Text-fig. 1, to show the character of the terminal tufts and the ovules on the inner sides (BENSON)
3. (*Calathiops Gothani*, n. sp.). Drawings from the two counterparts of the specimen showing the younger phase of a species somewhat similar to *C. Bernhardti* (BENSON)
- Transverse section of the stem of *Alstroemeria*, shown in plan to illustrate the

Index.

RES.	PAGE
distribution of the bundles and the position of the zone of lignified cells (PRIESTLEY, SCOTT, and GILLETT)	163
2. Part of the transverse section of an adult internode (PRIESTLEY, SCOTT, and GILLETT)	163
3. A vegetative shoot of <i>Alstroemeria</i> (PRIESTLEY, SCOTT, and GILLETT)	164
4. Diagram to show the course of the leaf-trace bundles in the stem (PRIESTLEY, SCOTT, and GILLETT)	165
5. Diagram showing the course of some of the longitudinal veins in the leaf (PRIESTLEY, SCOTT, and GILLETT)	169
6. Diagram showing the position of linkages of median strands in the orthostichy bearing leaves 1, 9, and 17 (PRIESTLEY, SCOTT, and GILLETT)	171
7. Plan of the apex showing the origin of primordia (PRIESTLEY, SCOTT, and GILLETT)	173
8. Diagram showing the conception of the single unit of shoot growth in <i>Alstroemeria</i> (PRIESTLEY, SCOTT, and GILLETT)	175
9. Diagram showing the arrangement of the foliar regions of the units of shoot growth in the stem of <i>Alstroemeria</i> (PRIESTLEY, SCOTT, and GILLETT)	175
10. The arrangement of the primordia in cross section of the bud of <i>Alstroemeria</i> (PRIESTLEY, SCOTT, and GILLETT)	176
11. (a) Diagram illustrating the theoretical transition from 1/3 to 2/5 phyllotaxis. (b) The transition from 2/5 to 3/8 (PRIESTLEY, SCOTT, and GILLETT)	177
1. Graph showing the relation between the growth-rates (DICKSON)	183
2. Front- and side-view drawings showing the construction of the bifilar micrometer used in measuring growth-rates (DICKSON)	184
3-7. Diagram showing typical examples of the different types (DICKSON)	187
8-14. For explanation see Text (DICKSON)	190-5
1. <i>Oliveria terrestris</i> sp. nov. <i>a</i> , long-celled filaments, giving rise to short-celled filaments; <i>b</i> ; <i>c</i> , club-shaped terminals; <i>d</i> , swollen portion due to localized division of some of the cells (NAYAL)	206
2. <i>O. terrestris</i> sp. nov. Thick-walled cells (akinetes) producing long-celled filaments in agar cultur (NAYAL)	207
3. <i>O. terrestris</i> sp. nov. A, formation and escape of swarmers; B, biciliate zoospore; C, quadriciliate zoospore; D, gamete; E, fusion; F, resting spore; G-L, various stages in the germination of zoospores (NAYAL)	208
4. <i>O. terrestris</i> sp. nov. Aerial brown-coloured filaments with thick walls showing branching, cell groups, and club-shaped ends (NAYAL)	209
5. <i>Pseudoleptosira calcarea</i> sp. nov. A, complete specimen showing habit and rhizoidal cell; B, cells dividing into a number of reproductive units (zoospores ¹); C, empty cells with apertures through which reproductive units escaped; D, a small branch with akinetes; other cells showing structure, each with a single pyrenoid (NAYAL)	211
1. Diagram to show the relations between the transpiring cells of the leaf and the conducting tracts of the stem (HAINES)	216
2. Diagram of elevation of pressure cylinder with lifting gear and accessories (HAINES)	220
3. Graph of results of Experiment 21, Table IX, showing changes in absorption rate on alterations of the pressure deficit (HAINES)	235
1. Camera-lucida outlines of a series of transverse sections of leaflets (HOLDEN)	241
2. A. Diagram of oblique section of leaflet indicating position of stomata. B. Three stomata from the same section (HOLDEN)	242
1. The effect of growth and ringing upon the volume-weight of apple wood. 1929 experiments (LOOMIS)	251
2. Growth and composition of large ringed branches in per cent. of normal (LOOMIS)	252
3-5. 3. Carbohydrates of the bark of the base segment. 4. Carbohydrates of the bark of the middle segment. 5. Carbohydrates of the bark of the upper segment (LOOMIS)	252

FIGURES.	PAGE
18. Very young cystocarp, showing the development of the first carposporangia (DREW)	447
19. Filament bearing two mature cystocarps (DREW)	447
1. Tamarix. Surface section of scale-leaf (SMITH)	453
2. Leucadendron. Surface section of lower epidermis (SMITH)	455
3. Trientalis europea. Stomata parallel with sides of the rectangle (SMITH)	457
4. Buxus. Young leaf. Stomata and veins near midrib (SMITH)	458
5. Buxus. Young leaf. Stomata and veins at edge of lamina (SMITH)	458
6. Complete strip obtained from the region between two lateral veins (SMITH)	459
7 A and B. Sambucus nigra. Stomata and veins of a shade-leaf (SMITH)	460
8. S. nigra. Stomata and veins of a sun-leaf (SMITH)	461
9. S. nigra. Apex of very young leaf showing high correlation between the stomatal orientation and the midrib (SMITH)	462
10. Ficaria verna. Plan of leaf (SMITH)	464
11. F. verna. Detail of area no. 5, Fig. 10 (SMITH)	465
12. F. verna. Young leaf (SMITH)	466
13. F. verna. Mature leaf (SMITH)	468
14. Graph showing stomata classified into 'angle-groups' (SMITH)	470
15. Graph showing stomata classified into 'angle-groups' (SMITH)	471
1. Copy of drawing of <i>H. rotundifolius</i> published by J. B. à Stapel in 1644 (WICKS)	495
2-3. <i>H. coccineus</i> . Lines drawn through points with the same stomatal frequency values. 2. Lower surface. 3. Upper surface (WICKS)	496
4-5. <i>H. coccineus</i> . Lines drawn through points with the same stomatal index values. 4. Lower surface. 5. Upper surface (WICKS)	497
6-7. <i>H. coccineus</i> . 6. Stomatal frequencies for upper and lower surfaces. 7. Stomatal indices for upper and lower surfaces (WICKS)	499
8. <i>H. rotundifolius</i> . Longitudinal section of a papilla (WICKS)	501
9-10. <i>H. rotundifolius</i> . Epidermis from the base of the leaf for the lower and upper surfaces respectively (WICKS)	502
11. <i>Brunsvigia gigantea</i> . Papilla in longitudinal section (WICKS)	504
1. <i>Eucalyptus macrorrhyncha</i> . Shedding of branches by gummosis. A. Side branch living B. Side branch two years later. C. Three years later. Branch shed. D. Five years later. Wound healed and cavity filled with new wood (EWART)	508
1-6. <i>Phylloglossum Drummondii</i> , Kunze. Gametophyte. 1. Crown with antheridia and shaft, in median longitudinal section. 2. Fungal tubercle in median section. 3. Antheridium with sperm cells. 4. Cap cell of antheridium, surface view. 5. Antheridium in median longitudinal section. 6 a and b. Details of fungus in tubercle cells (HOLLOWAY)	515
1. Hypothetical curves to show expected variations in absorption rate with increasing saturation of tissues and decreasing resistance to flow (HAINES)	534
2. Curves showing the relation between absorption rate and pressure deficit with increasing deficits plotted from the figures in Table XIV (HAINES)	542
3. Curves showing initial and final or minimum and maximum rates of absorption under different sustained constant deficits (HAINES)	545
4. Recovery curves for <i>Acer</i> (HAINES)	549
5. Recovery curves for <i>Aesculus</i> (HAINES)	552
6. Illustrating the mechanism of the recovery effect (HAINES)	560
7. Curve showing the relation which would be expected to exist between conduction rate and pressure deficit on the assumption that the only changes taking place in the resistance to flow would be those naturally accompanying different rates of flow as a result of the fact that the resistance is due to the viscosity of a flowing liquid (HAINES)	563
1. Section of apparatus used (BURGESS)	573

FIGURES.	PAGE
1. Diagram to illustrate conflicting interpretations of the structure of a chromatid at anaphase and telophase of mitosis and the first meiotic division (DARLINGTON)	581
1-3. Type A (Leaves of <i>Lepidodendron veltheimianum</i>). 1. Transverse section. 2 and 3. Surface sections of epidermis and stomata (GRAHAM)	590
4-7. Type B (<i>Lepidophyllum minor</i>). 4. Transverse section. 5, 6, and 7. Surface sections of epidermis, stomata, and of sub-stomatal hypoderm (GRAHAM)	590
8-10. Type C. 8. Diagrammatic transverse section. 9. Detail of bundle. 10. Surface section of stomata (GRAHAM)	590
11-15. Type D (<i>Lepidophyllum Sewardi</i>). 11. Diagrammatic transverse section. 12. Diagrammatic transverse section. 13. Transverse section. 14. Sieve-tube? 15. Surface section of stomata (GRAHAM)	590
16-23. Type E (<i>Lepidophyllum latifolium</i>). 16. Diagrammatic transverse section. 17. Transverse section. 18. Longitudinal section. 19 and 20. Surface sections of epidermis and of stomata. 21 and 22. Surface and longitudinal section of sub-stomatal hypoderm. 23. Transverse section of stomata and sub-stomatal hypoderm (GRAHAM)	592
24-28. Type F (<i>Lepidophyllum equilaterale</i>). 24. Diagrammatic transverse section. 25. Transverse section of stoma. 26. Transverse section of leaf. 27 and 28. Surface sections of stomata and of sub-stomatal hypoderm (GRAHAM)	592
29-30. Type G (Leaves of <i>Lepidodendron esnotense</i>). Diagrammatic transverse sections (after Renault's figures). 29 near apex, and 30 near base of leaf (GRAHAM)	592
31-37. Type H 1 (<i>Lepidophyllum Thomasi</i>). 31. Diagrammatic transverse section. 32. Transverse section. 33. Longitudinal section. 34. Surface section of stomata. 35. Thin-walled cells of 'phloem'. 36. Thick-walled cells of 'phloem'. 37. Transverse section of stomata and underlying hypoderm (GRAHAM)	594
38. Type H 2. Diagrammatic transverse section (GRAHAM)	594
39-40. Type H 3 (<i>Lepidophyllum papillonaceum</i>). 39. Diagrammatic transverse section. 40. Transverse section of same (GRAHAM)	594
41-42. Type I. 41. Diagrammatic transverse section. 42. Transverse section of same (GRAHAM)	594
43. Type J (<i>Lepidophyllum angulatum</i>). Diagrammatic transverse section (GRAHAM)	594
44-45. Type J (<i>Lepidophyllum angulatum</i>). 44. Diagrammatic transverse section. 45. Transverse section (GRAHAM)	598
46-49. Type K (<i>Sigillariopsis sulcata</i>). 46. Diagrammatic transverse section. 47. Diagrammatic transverse section. 48. Transverse section. 49. Transverse section of stroma (GRAHAM)	598
50-51. Type L (<i>Sigillariopsis halifaxensis</i>). 50. Diagrammatic transverse section. 51. Transverse section of same (GRAHAM)	598
52-53. Type M (Leaves of <i>Sigillaria Brardi</i>). Diagrammatic transverse sections after figures by Renault. 52, near base, and 53, near apex (GRAHAM)	598
1. Material collected on 22 April 1934 (HAAS, HILL, and KARSTENS)	610
2. Material collected on 28 May 1934 (HAAS, HILL, and KARSTENS)	611
3. Material collected on 1 July 1934 (HAAS, HILL, and KARSTENS)	612
4. Material collected on 12 September 1934 (HAAS, HILL, and KARSTENS)	613
5. Seasonal variation in calcium and magnesium carbonates (HAAS, HILL, and KARSTENS)	615
6. Mean percentage values of total nitrogen of thallus, total N of extract, amino N, amide N, and galactose (HAAS, HILL, and KARSTENS)	617
1. Carpospores of <i>Lemanea torulosa</i> , early stages of germination (ROSENBERG)	621
2. Young sporelings of <i>Lemanea torulosa</i> (ROSENBERG)	621
1-8. Gametogenesis in living material. 1. Hyphal tip vitally stained with Janus green showing distribution of chondriosomes and lipid granules. 2 a, b, c, d. Stages in gametangial cross-wall formation. 3. Male and female gametangia. The 'granular' stage of gametogenesis. 4. Male and female gametangia.	

FIGURES.

PAGE

	The 'gamete origin' stage of gametogenesis. 5. 'Gamete origin' stage showing relative number and size of nuclei in male and female gametangia. 6. 'Disappearance' stage. 7. Cleavage. 8. Male and female gametes each with a nucleus, a nuclear cap, and lipoid granules (HATCH)	628
9-15.	Nuclear phenomena in the hyphal tip as demonstrated with the Feulgen reaction for chromatin. 9. Hyphal tip showing distribution of nuclei. 10. A resting nucleus. 11. Early anaphase, side view. 12. Early anaphase, polar view. 13. Anaphase, polar view. 14. Anaphase, side view. 15. Telophase (HATCH)	637
16-20.	Nuclear phenomena in the gametangia as demonstrated with the Feulgen reaction for chromatin. 16. Young gametangia but recently cut off from the hypha showing that the nuclear complement of the female gametangium at this time may even be greater than that of the male. 17. Anaphase, polar view. 18. Anaphase, polar view. 19. Telophase. 20. A female gamete (HATCH)	637
21-30.	Cytoplasmic phenomena of gametogenesis as demonstrated with Champy-Kull's chondriosomal technique. 21 and 22. Segments of the hypha. 23. 'Granular' stage of gametogenesis. 24. 'Gamete origin' stage. 25. Female gametangium, 'disappearance' stage. 26. Female gametangium, cleavage completed. 27. A gamete from a female gametangium. 28. Gametes from a female gametangium. 29. Gametangia and insert from female gametangium. 30. Male and female gametes (HATCH)	640
31-3.	31. Insert from a female gametangium. 32. Female gametangium showing the orientation of gametes. 33. Monster with three nuclear caps fused, three nuclei free, and three cilia (HATCH)	642
1.	Diagram showing the structure of the internode and wound cork formation round a wounded part of the cortex (BLOCH)	653
2.	Transverse section of an internode showing the wound-margin with cell divisions in epidermis (BLOCH)	654
3.	Surface section to show the distribution of cell divisions in the epidermis (BLOCH)	655
4.	Transverse section of an internode below node 5, showing meristematic activity in a wounded area caused by scratching (BLOCH)	657
5.	Transverse section of an internode showing meristematic activity above a wound (BLOCH)	657
6.	Small portion of Fig. 2 showing in the centre a parenchyma cell which has become thickened and lignified after wounding (BLOCH)	658
7.	Appearance of the sclerenchyma sheath in a region of vigorous meristematic activity in a young internode (BLOCH)	659
8.	Transverse section of a medullary bundle at considerable distance from the wound (BLOCH)	660
9.	The same bundle immediately bordering on the wound meristem, and connected up with another bundle by thickened and lignified pith cells (BLOCH)	661
10.	Transverse section through the region of two medullary bundles two months after wounding, showing both meristematic activity of the sheath cells and formation of thickened and lignified cells (BLOCH)	662
11.	Transverse section of an internode showing an area consisting of thickened pith parenchyma cells surrounded by cell divisions in the neighbourhood of a wound caused by pricking (BLOCH)	664
1.	Equivalent portions of the leaf apex from leaves of OO and oo individuals (ANDERSON and DE WINTON)	674
2.	Equivalent portions of the calyx of crimp (ff) and non-crimp (FF) to show differences in shape and venation (ANDERSON and DE WINTON)	677
3.	Comparison of calyx lobe development in crimp (ff) and non-crimp (FF) (ANDERSON and DE WINTON)	677
4.	Growth curves of corolla limb in crimp (ff) and non-crimp (FF) (ANDERSON and DE WINTON)	677
5.	Diagrams of inflorescences of chch and ChCh plants (ANDERSON and DE WINTON)	679

FIGURES.

PAGE

6. Equivalent portions of the calyx from *chch* and *ChCh* individuals (ANDERSON and DE WINTON) 679
7. Epidermal cells of *PP* and *pp* individuals, viewed from the side (ANDERSON and DE WINTON) 680
8. Leaf, bract, calyx, and corolla of the 'wild' type of *P. sinensis*, and of nine mutants affecting leaf and inflorescence shapes (ANDERSON and DE WINTON) 681
9. Diagram showing the effect of the mutant genes 'oak' and 'sinensis' on the lower and upper whorls of bracts of 'leafy calyx' plants (ANDERSON and DE WINTON) 682
10. Number of similar reactions to mutant genes between leaf and corolla, leaf and calyx, &c. (ANDERSON and DE WINTON) 686
- 1-4. Side-views of first metaphase in pollen mother-cells. 1. With association of four chromosomes giving half-disjunction at anaphase. 2. With chain of four giving numerical non-disjunction. 3. With association of four giving non-disjunction, homologous centromeres directed towards the same pole. 4. With seven bivalents (SUTTON) 691
5. Diagram illustrating half-disjunction (SUTTON) 693
6. Associations of four chromosomes from different pollen mother-cells at first metaphase (SUTTON) 694
7. Diagram of chromatid structure of associations in Figs. 1-3 and Fig. 6; *a-c* correspond to Figs. 1-3, *d-f* represent Fig. 6 *a-g* (SUTTON) 695
1. A. Seedling of *P. sylvestris* at early stage of infection. B. Two primary leaves, on left young, on right older, stage of disease with spermogonia. C. Spots of early infection on a double needle as in A. D. A double needle with mature apothecia. E. Two mature apothecia at the leaf angles. F. A dehiscent apothecium (JONES) 701
2. Stages of ascospore germination (JONES) 703
3. A. Transverse section of a stoma. B. The invading hypha within stomatal pit surrounded by expanded gelatinous sheath (JONES) 705
4. Portion of transverse section of leaf showing mycelium in the disintegrated mesophyll (JONES) 706
5. A. The black rings or diaphragms dividing the leaves into zones. B. Perspective diagram of inset portion of A, showing that the diaphragms are only partial and interrupted by endodermis. C. Portion of a radial leaf section showing the fungal vesicles in the sub-stomatal cavities (JONES) 707
6. A. Longitudinal section of basal part of a dwarf shoot infested with fungus and dense deposit of black substance at base. B. Radial longitudinal section of a young stem invaded by fungus from seat of such a dwarf shoot as A. C. Longitudinal section of basal part of a dwarf shoot infested with fungus but with normal cork-layers causing fall of shoot (JONES) 709
7. Transverse section of a portion of leaf about to form a fructification (JONES) 710
8. Transverse section of a small spermogonium (JONES) 712
9. A mature spermogonium with marginal dehiscence (JONES) 712
10. Portion of a spermogonium in transverse section (JONES) 713
11. A-E. Oogonial cells all binucleate. F-J. Spermatophores (JONES) 714
12. A, B. Transverse section of young apothecia accommodated within spermogonia (JONES) 715
13. A. Transverse section showing the limits of an apothecium, formed independently of a spermogonium, and removal of epidermal lamellae on a wide scale. B. Early stage of apothecium development (JONES) 716
14. A. Further early stage in apothecium development. B. The denser cells picked out from the above drawing and contents of remaining tissue omitted (JONES) 717
15. Transverse section of a young apothecium showing prominence of ascogonial cells (JONES) 718
16. Portion of transverse section of young apothecium showing formation of ascogenous hyphae from the multinucleate ascogonial cells which occur singly or in short coils (JONES) 719

FIGURES.

PAGE

17. A. Transverse section of apothecium forming ascogenous hyphae. B. Portion of hymenium showing formation of asci (JONES)	720
18. A. Mature ascospores showing the single nucleus and highly granular contents. B. The nucleus in A. C. Portion of a transverse section of nearly ripe apothecium with asci at various stages of maturity (JONES)	721
19. Transverse section of a typical apothecium at time of active formation of asci (JONES)	722
20. A. Transverse section of a leaf with two mature apothecia. B. Portion of a mature apothecium at spore discharge. C. Transverse section of the median ridge of a roof showing the interlocking periphyses and mucilage within. D. The fissure of dehiscence (JONES)	723
1. Chart illustrating time of flowering of the treated hyacinths (HAVAS and CALDWELL)	740
1. Distribution curves of density for a number of alpine meadow species (BLACKMAN)	753
2. Distribution curves of density for <i>E. maritimum</i> in a number of similar associations (BLACKMAN)	753
3. Distribution curve of density (number of individuals per quadrat) for <i>P. auricula</i> (BLACKMAN)	755
4. Distribution curves of density for <i>P. media</i> (BLACKMAN)	755
5. Distribution curves of density for a number of species in the same association (Aberystwyth pasture) (BLACKMAN)	756
6. Distribution curves of density for <i>T. repens</i> in four different associations (BLACKMAN)	756
7. Distribution curves of density for <i>Agrostis</i> spp. and <i>F. ovina</i> occurring as dominant or subdominant species in three similar associations (BLACKMAN)	758
8. Distribution curves of density for some occasional species in the three associations observed (BLACKMAN)	758
9. The relationship between quadrat size and the average number of species found in the quadrat (BLACKMAN)	761
10. Graph showing the linear regression of the logarithm of percentage absence on density (BLACKMAN)	765
11. Scatter diagram demonstrating the correlation between the logarithm of percentage absence and the mean percentage area covered by <i>T. repens</i> (BLACKMAN)	765
12. The relationship between the logarithm of percentage absence and 'abundance' (a measure of density) deduced from published data by McGinnies (1934) for some semi-desert associations (BLACKMAN)	771
13. The upper diagram gives the variation in the information respecting density which is available from noting the percentage absence as the percentage absence changes with size of quadrat. The lower diagram gives the fraction this information is of the total information that would be obtained if the number of plants in each quadrat could have been counted (BARTLETT)	775
1. The distribution of species in percentage frequency classes (ASHBY)	782
2. The relation between percentage frequency and quadrat area (ASHBY)	783
3. The theoretical relationship between percentage frequency and mean area on the assumption of random distribution (ASHBY)	788
4. The range of mean areas corresponding to percentage frequency classes of equal width, on the assumption of random distribution (ASHBY)	789
5. Percentage frequency plotted against density in individuals per square centimetre for a population of <i>Salicornia</i> (ASHBY)	791
6. Natural logarithms of the chance of <i>not</i> finding an individual in a quadrat 25 square cm. in area, plotted against density of <i>Salicornia</i> in individuals per square cm., together with the theoretical line on the assumption of random distribution (ASHBY)	792
7. Natural logarithms of the chance of <i>not</i> finding an individual in a quadrat 100 square cm. in area, plotted against density of <i>Salicornia</i> in individuals per square cm., together with the theoretical line on the assumption of random distribution (ASHBY)	793

FIGURES.

PAGE

8.	Number of empty quadrats in a lattice of 25 thrown on 100 areas of known density, plotted against the density, together with the theoretical curve (ASHBY)	794
9.	Number of empty quadrats in a lattice of 25 thrown on 68 areas of known density, plotted against the density, together with the theoretical curve (ASHBY)	795
1.	Knight's porometer with 'constant level' attachment (BOLAS and SELMAN)	804
2.	Knight's porometer and the recording apparatus (BOLAS and SELMAN)	805
3.	Trip device for obtaining a twenty-four-hour record with a twelve-hour clock (BOLAS and SELMAN)	805
4.	Circuit diagram (BOLAS and SELMAN)	806
5.	Graph of bubble frequency plotted against time of day (BOLAS and SELMAN)	806
6.	Portion of a twenty-four-hour stomatal record (BOLAS and SELMAN)	807
1-3.	<i>Woodfordia floribunda</i> . Fig. 1, a normal embryo-sac showing two synergids, two polar nuclei and three antipodals. Fig. 2, a part of the same at a later stage showing two synergids and the two polar nuclei. Fig. 3a, an ovule showing an embryo-sac with reversed polarity. Fig. 3b, an enlarged sketch of the embryo-sac of the same (JOSHI and VENKATESWARLU)	842
1-6.	1. Diagram of apparatus used. 2. Portion of the ventral surface of the thallus of <i>Fimbriaria bleumeana</i> showing location and arrangement of the scales and the investing mass of rhizoids. 3. Diagram of a section of the thallus and part of the stalk of the carpocephalum of <i>Fimbriaria bleumeana</i> showing the rhizoidal groove with rhizoids, and the multicellular hairs on the stalk of the carpocephalum. 4. Portion of the above drawn in detail to show the tuberculate rhizoids of the groove and the nature of the hairs. 5. Thallus of <i>Fimbriaria bleumeana</i> bearing stalked carpocephalum showing the distribution of hairs on the stalk and amongst the rays of the head. 6. Diagram of a section of the head of the carpocephalum showing the location of the archegonia surrounded by the multicellular hairs (BOWEN)	845
1-5.	<i>Filipendula Ulmaria</i> Maxim. 1. Preparation of the stem apex of a K_4C_4 flower with six ovaries, of which three have been removed; the remaining three are seen overlying the tissue of the flower wall. 2. Transverse section of a K_4C_4 flower with three ovaries. 3. Transverse section of the same gynaecium after the appearance of the third loculus showing the midrib of this ovary cut twice as seen in the older ovaries of 2. 4. Transverse section of one of the ovaries seen in 2 and 3 showing the midrib and placental bundles 'out of line' owing to torsion. 5. Preparation of a single ovary showing the one entering bundle from which the twin placental strands are derived later (SAUNDERS)	849
6-11.	<i>Filipendula hexapetala</i> Gillib. 6. Preparation from a K_7C_7 flower with thirteen ovaries showing the trunk cords for sepals, petals, and stamens as in 5. 7. Preparation of a K_8C_8 flower with ten ovaries showing the origin of the bundles for two neighbouring ovaries from a common point. 8. Preparation of a gynaecium of eleven ovaries. 9. The vascular system of the same gynaecium enlarged showing a kink in the course of the bundles at the point at which they turn outwards. 10. Preparation of the stem apex of another flower with two attached ovaries showing the origin of the twin placental strands from the single entering bundle. 11. Longitudinal section of an ovary showing the midrib bundle undifferentiated in the basal region and hence appearing disconnected from the placental strands (SAUNDERS)	851
1.	Diagrammatic section through the dish with moat and perforation lid (DOBBS)	853
2.	A rectangular Petri dish which can be placed lid downwards on the stage of the microscope and gripped by the mechanical stage (DOBBS)	853

The Cytology of the Mushroom *Psalliota campestris* Quel.

BY

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With forty-nine Figures in the Text.

IT has long been known that the edible mushroom *Psalliota campestris*, like several other Basidiomycetes, exists in two varieties (3), (27), one with four spores and one with two. Both varieties are found growing wild in Europe and America, but it is the two-spored form which is now in cultivation.

Although the existence of two varieties is common knowledge there seems to be a lack of certainty as to the nuclear phenomena associated with spore formation. For the four-spored Basidiomycetes which have been investigated cytologically, the results in general are, that the basidium is four nucleate just before spore formation and that each spore as it develops receives one of these basidial nuclei. For the two-spored Basidiomycetes, including both the habitually two-spored ones and the two-spored varieties of four-spored forms, the position is much less simple, and many of the theoretical possibilities have been described for actual species. Giving names of species as illustrative examples only, the position may conveniently be summarized in the following way:—

1. Four-nucleate basidium, each spore receiving only one basidial nucleus, the other two degenerating in the basidium e.g., *Amanita bispora* (19), *Mycena galericulata* (5), *P. campestris* (5), *Craterellus cornucopioides* (5).

2. Four-nucleate basidium, each spore receiving two basidial nuclei, e.g. *P. campestris* (27), *C. cornucopioides* (25), *Hydnangium carneum* (26).

3. Four-nucleate basidium, each spore receiving two basidial nuclei, one of which subsequently migrates back into the basidium, e.g. *Dacryomyces chrysocomus* (12), *D. deliquescens* (13).

4. Two-nucleate basidium, each spore receiving one basidial nucleus, e.g. *M. galericulata* (16), *D. deliquescens* (6).
5. Eight-nucleate basidium, each spore receiving one basidial nucleus, the other six degenerating in the basidium, e.g. *Clavaria cristata* (4), *C. cineria* (4).

It will be seen at once, even from the few names quoted, that some of the species, including *P. campestris*, have been cited as examples of more than one type of development. With regard to *P. campestris* in particular, several authors have given accounts of spore formation. Maire (20) in 1902 described it for the two-spored variety, stating that two basidial nuclei enter each spore. Lewis (19) in 1902 also examined *P. campestris* with a view to confirming his observations of nuclear behaviour in *Amanita bispora* (19). In the latter he found that only one basidial nucleus entered each spore (see 19, p. 2), but he stated that the nuclei of *P. campestris* were too small to yield a satisfactory result. In 1928 Sass (27) described spore formation for the two-spored variety of *P. campestris*, stating that the spores are initially binucleate, each spore receiving two of the four basidial nuclei. Buhr (5), in 1932, while knowing of the existence of the earlier paper by Sass, stated that, in common with six other bispored forms, the spores of the two-spored variety of *P. campestris* are initially uninucleate, each receiving but one of the four basidial nuclei. No account has been given of spore formation in the four-spored variety of *P. campestris*.

Since an adequate account of the morphology and development of the fruit body of the mushroom has been given by more than one investigator (1), (2), (9), this part of the life-history has not been redescribed. In addition to the work on morphology and spore formation an investigation of a different nature was undertaken by Hirmer (11) in 1920. This work must be mentioned here since it probably has an indirect bearing on the present problem and will be referred to again later. In this paper Hirmer described a multinucleate mycelium which he obtained by means of tissue cultures from the pileus of *P. campestris*. He stated that, when cell division was about to take place, one or more of the nuclei of that cell failed to divide. The newly formed cell in this way contained fewer nuclei than its parent. By this method the number of nuclei in the cells of the mycelium was gradually reduced, as growth continued, until the cells at the tips of the youngest hyphae contained only two nuclei. This work has not been repeated.

The present work is an attempt to give as complete and connected an account as possible of the nuclear behaviour of both forms of *P. campestris*. Special attention has been paid to the two-spored form since this is the more controversial part of the investigation.

MATERIAL AND METHODS.

Collections and fixations of two and four-spored mushrooms were made during the spring and summer of 1933. The two-spored form was obtained from a local nursery garden,¹ fixations being made on the spot. Collections of the wild mushroom were made from six widely separated localities in England; all the plants from these localities were found to possess four spores. Most of the material for the study of the four-spored variety was also fixed at once in the field, but two fixations were made from material which had been sent by post. In these two cases it was found that, although nuclear divisions were still to be seen, the general effect of the fixation was far less satisfactory than that of material fixed as soon as it was gathered. The exceptionally dry summer of 1933, which prevented rapid growth, considerably increased the difficulty of obtaining material of the four-spored form which was as well fixed as that of the cultivated variety, where the external conditions were under control.

Fructifications having an unbroken veil were fixed for stages up to the formation of the definitive nucleus of the basidium, those fully expanded with the veil just broken were found to possess all stages from young basidia to those bearing ripe spores. The fixatives used were Flemming's strong fluid diluted to half strength by the addition of an equal quantity of water and La Cour's 2BD (17). Both these fixatives gave satisfactory results. For all fixations an exhaust pump was used to insure the best possible penetration of the fixing fluid. The fixed material was embedded and cut into sections of from 4 to 12 μ thick. Most of the sections were stained in Heidenhain's haematoxylin, but a few were stained in Flemming's triple stain. Where information as to the number of nuclei per cell only was required, Delafield's haematoxylin was used. The mycelium from the spores and tissue cultures was examined alive in water, stained in iodine and mounted in water and also stained in Heidenhain's haematoxylin and mounted in glycerine jelly.

I wish to take this opportunity of thanking Mr. Brundrett for his very kind permission to use unlimited material from his nursery garden, Mr. Holmes Smith for his information as to local growers, Mr. Winter for collection and fixation of some of the wild material, and Professor Lang for help and criticism during the course of the work.

¹ Mature specimens of cultivated varieties of *P. campestris* and *P. arvensis* are sometimes similar in external appearance. The cultivated material used in this investigation was from one source only, was watched over a long period of growth and seen in many stages of development. It conformed in every respect to the descriptions (i.e. gill colour during growth, form of ring, &c.) given for *P. campestris* Quel. and was in addition to this uniformly two-spored (23).

THE NUCLEI OF THE FRUCTIFICATION AND THE YOUNG BASIDIUM.

The early stages of development of the mushroom are, as far as can be ascertained, exactly the same in the two varieties. The account in this section will therefore refer to both forms unless a definite statement to the contrary is made. The figures are taken mainly from preparations of the two-spored material; comparable stages have been seen for the four-spored variety, but it has not been thought necessary to duplicate every figure.

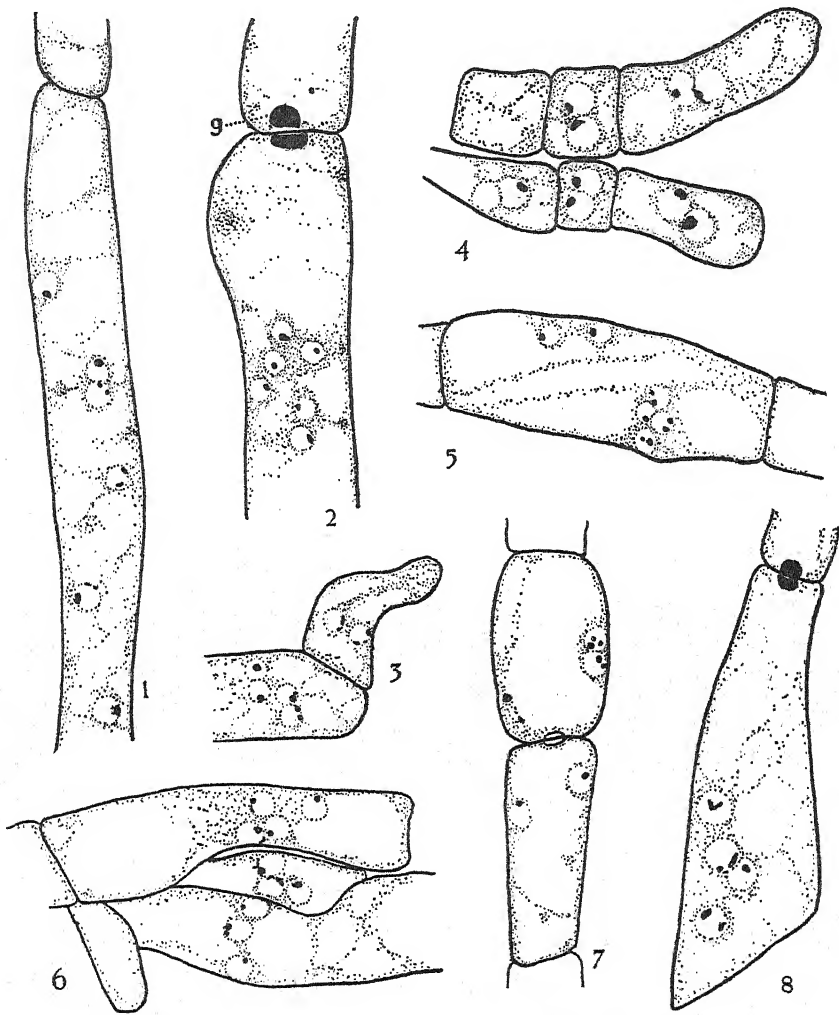
The youngest fructifications examined were very small undifferentiated wefts of hyphae about two millimetres in diameter, to the base of which there remained attached strands of hyphae which had been in connexion with the subterranean mycelium. All the cells of the hyphal strands and of the young fructifications are long, narrow, and multinucleate (Figs. 1 and 2). The nuclei are from three to ten in number and may be scattered along the length of the cell or collected in a group against the cell-wall if the cytoplasm is scanty. The cross-walls of most of the hyphae are rendered the more conspicuous by the presence of a stainable hemispherical granule on each side of the septum (Fig. 2).

By the time the young fructifications have become about a centimetre in diameter the differentiation into stipe, pileus and gill has begun, and information as to the number of nuclei in the cells in each region has been obtained. The cells of the stipe and pileus are multinucleate, the former containing from six to ten nuclei (Fig. 3) those of the latter more often four to six. The cells at the edges of the gills, that is the basidia, and the two or three cells immediately behind the basidia (i.e. the sub-hymenial layer) are all binucleate (Fig. 4). A few complete cells of the trama have been seen, some containing four nuclei, some two. The numbers of nuclei obtained by these counts suggest that a process of gradual reduction, such as was deduced by Hirmer (11), does actually take place in the hyphae of the fructification. The solution of the problem as to exactly how and where the change occurs could only be obtained by following the course of individual hyphae from the basidium to the pileus, counting the nuclei of each successive cell of the hypha. This, owing to the crowding and twisting of the hyphae of the trama, is not possible.

In fully expanded fructifications, the cells of the stipe (Fig. 6) are still multinucleate, containing from six to eight nuclei, those of the pileus from four to six nuclei (Fig. 5), while those of the trama are usually binucleate (Fig. 7). Some cells of the trama, however, have been seen which contain three or four nuclei (Fig. 8). The cells of the sub-hymenial layer and the young basidia are binucleate (Fig. 9).

The young basidium remains binucleate for some time and increases considerably in size. The nuclei of all the cells up to the binucleate

basidium are very small (Fig. 1) and contain very little stainable material apart from the nucleolus. The two nuclei of the basidium, which are but



FIGS. 1-8. 1. Portion of cell of mycelial strand connecting fructification to subterranean mycelium, six nuclei shown. $\times 3,000$. 2. Part of cell of undifferentiated fructification, six nuclei shown. $\times 3,000$. 3. Parts of two cells of stipe of young fructification. $\times 2,100$. 4. Binucleate cells of sub-hymenium and basidia. $\times 3,000$. 5. Cell of pileus of mature fructification, six-nucleate. $\times 2,100$. 6. Multinucleate cell of stipe. $\times 2,100$. 7. Binucleate cell of trama. $\times 2,100$. 8. Four-nucleate cell of trama. $\times 3,000$. g = granule.

little larger ($1.4-1.6 \mu$) than those of the vegetative hyphae, now fuse and immediately the nuclear area of the fusion nucleus increases enormously in size ($4-5 \mu$). At the same time stainable chromatin material, in addition to the nucleolus, appears. At first the two nucleoli remain, lying separate

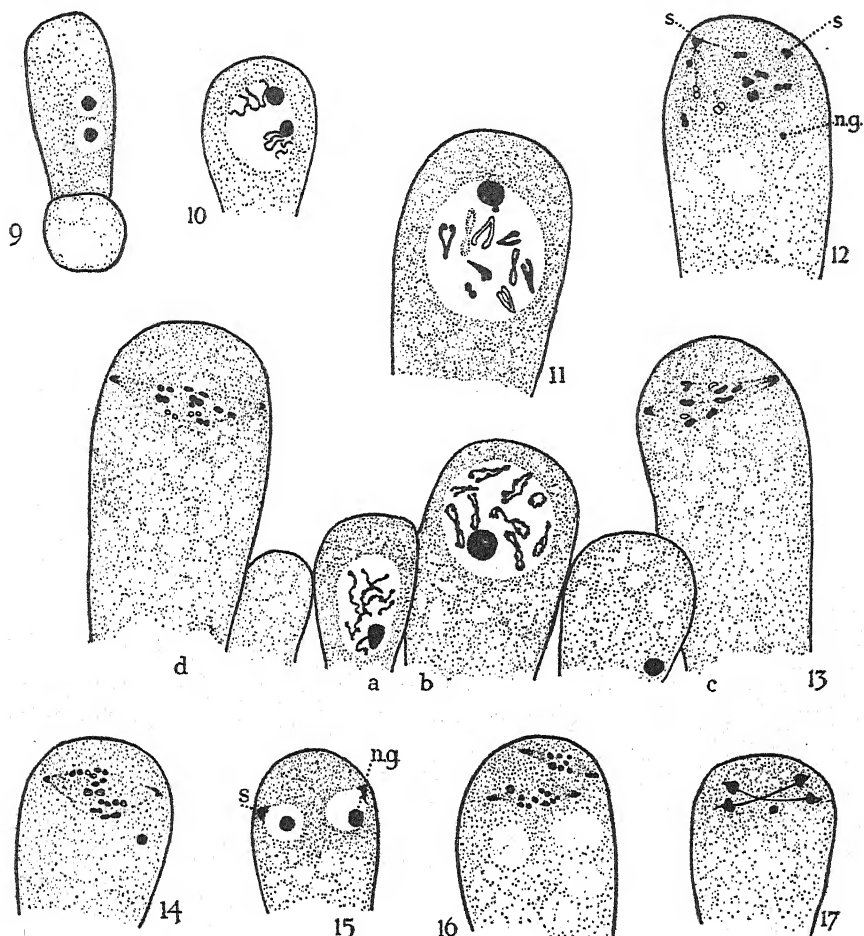
in the nuclear area (Fig. 10), but finally they also fuse forming the large deeply staining nucleolus of the definitive nucleus of the basidium (Fig. 13 *a*).

The definitive nucleus remains for some time nearly filling the swollen end of the now clavate basidium before it undergoes division. The chromatin, which is well marked at this stage, appears to be either in the form of a continuous thread or of long twisted but separate chromosomes (Fig. 13 *a*). At early diakinesis seven to nine very long gemini can be seen (Fig. 13 *b*) which then shorten and thicken and so assume the very characteristic shapes seen at a later stage (Fig. 11). Nine distinct pairs of chromosomes can now be seen within the nuclear area in addition to the nucleolus. The next recognizable phase is a very late diakinesis. The nuclear area has quite disappeared, the nine gemini are now very small and occupy a roughly triangular area at the top of the basidium (Figs. 12 and 31). One of the apices of the triangle is occupied by one of the gemini, the other two by small stainable granules, the centrosomes (Figs. 12 and 31, *s*). Spindle 'fibres' are just visible radiating from the centrosomes. Some of the chromosomes have already reached a central position on the spindle, others seem to be moving towards it. The nucleolus (Fig. 12) is sometimes still visible outside the triangular area of chromosomes and spindles. This appearance, although it may represent but a transient condition in life is frequently to be seen in fixed material. Stages showing the formation of the centrosomes, such as those described by Wager (33) have not been found.

The spindle now becomes organized as a narrow structure stretching across the basidium at right-angles to its long axis. The chromosomes lie scattered along the length of the spindle (Figs. 13 *c* and 32) and the pairs tend to separate at different times so that during early anaphase anything from about twelve up to eighteen chromatin bodies may be visible (Figs. 13 *d* and 14). Taking into account all the stages of nuclear division examined it will be seen that the diploid number of chromosomes for *P. campestris* is eighteen and the haploid nine; a larger number than has been previously recorded for a Basidiomycete. Late anaphases can frequently be found where the chromosomes are lying close together near the spindle ends, in this position it is impossible to see more than four chromatin masses at each end; these masses represent several, not single, chromosomes. This appearance of the chromosomes at anaphase will be referred to again later.

After the first division the two daughter nuclei become reorganized, each now appearing as a small clear area in the cytoplasm (Fig. 15). Each nucleus contains a nucleolus and has its chromatin in a mass near the centrosome which persists for a short time after division, making the nucleus slightly beaked. The nuclei have once more become too small to

allow of the examination of the early stages of division. The next recognizable condition is that of metaphase of the second division where



FIGS. 9-17. 9. Young binucleate basidium. $\times 3,000$. 10. Nuclear fusion in basidium, two nucleoli present. $\times 3,000$. 11. Definitive nucleus of basidium, nine gemini and nucleolus. $\times 4,000$. 12. Late diakinesis with nine gemini. $\times 4,000$. 13. *a*. Prophase of first division in basidium. *b*. Early diakinesis, nine gemini present. *c*. Metaphase of first division, nine gemini. *d*. Early anaphase with seventeen chromosomes. $\times 4,000$. 14. Early anaphase with eighteen chromosomes. $\times 4,000$. 15. Binucleate basidium after first division. $\times 3,000$. 16. Metaphase of second division. $\times 4,000$. 17. Late anaphase of second division. $\times 3,000$. *s* = centrosome, *ng* = nucleolus. Differences of focal level of chromosomes are indicated by differences in shading.

six to nine chromosomes may be visible on the spindles. The two spindles of any one basidium are near the top of the cell and are more or less at right-angles to its long axis (Fig. 16). The chromosomes divide but are uncountable at anaphase (Fig. 17) and soon four small daughter nuclei become reorganized (Figs. 18 and 33). Each nucleus now has a clear nuclear area, a well-marked nucleolus and a very small speck of chromatin material.

Directly after formation these four basidial nuclei lie in a group near the tip of the basidium. The distance between the nuclei and the tip now increases, partly by growth in length of the basidium in preparation for the formation of the sterigmata and partly probably by the actual movement of the nuclei away from the tip. At this point there seems to be no signs of the centrosomes persisting, either near the nuclei or near the wall of the basidium (Figs. 18 and 33). The sterigmata make their appearance first as small bulges in the tip of the basidium (Figs. 19 and 34) and then as projecting horn-like structures, each bearing a very small spore (Figs. 20 and 35). Sometimes at this stage the narrowest region of one or both the sterigmata appears to contain a small quantity of stainable material, but since it is not constantly present, is located below the spore and is not present during the development of the sterigmata, it is probably a mechanical retention of the stain and not connected with the centrosomes (Fig. 20).

Several authors (18) (20) have described the occurrence of fibres connecting the basidial nuclei with the tip of the developing sterigmata and by contraction acting as a device for drawing the nuclei into the spores. These fibres have not been seen in either variety of mushroom, in spite of special efforts with the appropriate stain. (Flemming's triple stain, &c.).

From this stage onwards there is a difference of behaviour in the two varieties and spore formation in each variety will be described separately.

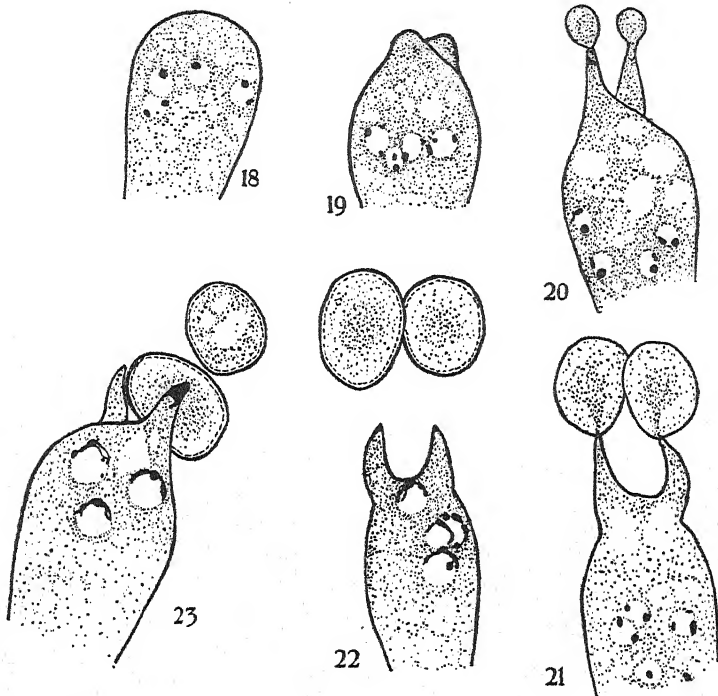
SPORE FORMATION IN THE TWO-SPORED VARIETY OF *P. CAMPESTRIS*.

In the two-spored variety of the mushroom only two sterigmata are formed, and each bears one spore (Fig. 20). These spores are at first very small, but they rapidly increase in size until they become very conspicuous objects (5 to 7 μ in diameter). They possess only a thin wall at first and they, together with the sterigmata and the top of the basidium, are densely filled with cytoplasm (Fig. 21). While the spores are developing the four nuclei which have been lying at the base of the basidium begin to move up towards the sterigmata. At this point the nuclei undergo a slight change in form, the stainable material increases in amount and becomes collected into a hemispherical mass at the upper side of the nucleus (Figs. 22 and 23).

The spore wall now becomes thickened all round except at the point of junction with the sterigma. By this time the upper two of the four basidial nuclei have reached the tip of the basidium. One nucleus then begins to pass through each sterigma. During their passage the nuclei quite lose their spherical shape and become greatly elongated. The nuclear area disappears from view and the chromatin becomes bent into a hair-pin shaped thread, the bend being directed towards the spore (Fig. 24). In this manner a nucleus makes its way into each spore where it retains its

elongated shape for a short time, looking somewhat like an inadequately fixed and stained dividing nucleus (Fig. 25).

The other two nuclei have by now reached the bases of the sterigmata (Fig. 24). One nucleus passes through each sterigma undergoing the same changes in shape as the first two (Figs. 25 and 26). In one case (Fig. 27)



FIGS. 18-23. Two-spored form. 18. Four-nucleate basidium. $\times 3,000$ 19. Young sterigmata. $\times 3,000$. 20. Young spores. $\times 3,000$. 21. Thin-walled spores, nuclei in base of basidium. $\times 3,000$. 22. Thick-walled spores, nuclei nearing sterigmata. $\times 3,000$. 23. Three nuclei in basidium, one in sterigma. $\times 3,000$.

the spores had become detached artificially during the process of mounting, but they show the second pair of nuclei in the act of entering them. It can be seen that the basidium contains no nuclei, each spore possesses one complete nucleus and part of a second; each sterigma also contains a portion of a nucleus. The nuclei had been broken across when the spores were pulled away. After the entrance of the second nucleus into each spore all four nuclei once more become spherical in shape (Fig. 28). The spores are therefore initially binucleate. All four nuclei now undergo division so that each spore becomes four-nucleate (Fig. 29). The spores as they ripen become dark in colour and opaque so that, in the majority of cases, it is not possible to tell whether the four nuclei undergo yet another division.

A few isolated spores have been found, however, where eight nuclei are to be seen (Fig. 30). This may be the normal number of nuclei for the mature spores, made visible by an accidental delay in the darkening of the wall. On the other hand, these spores might possibly be the product of very occasionally occurring monosporous basidia which had received all four basidial nuclei. Since no case of the passage of all four nuclei into one spore has been observed, and all the eight-nucleate spores have been detached and isolated, nothing more than a record of their occurrence can be made.

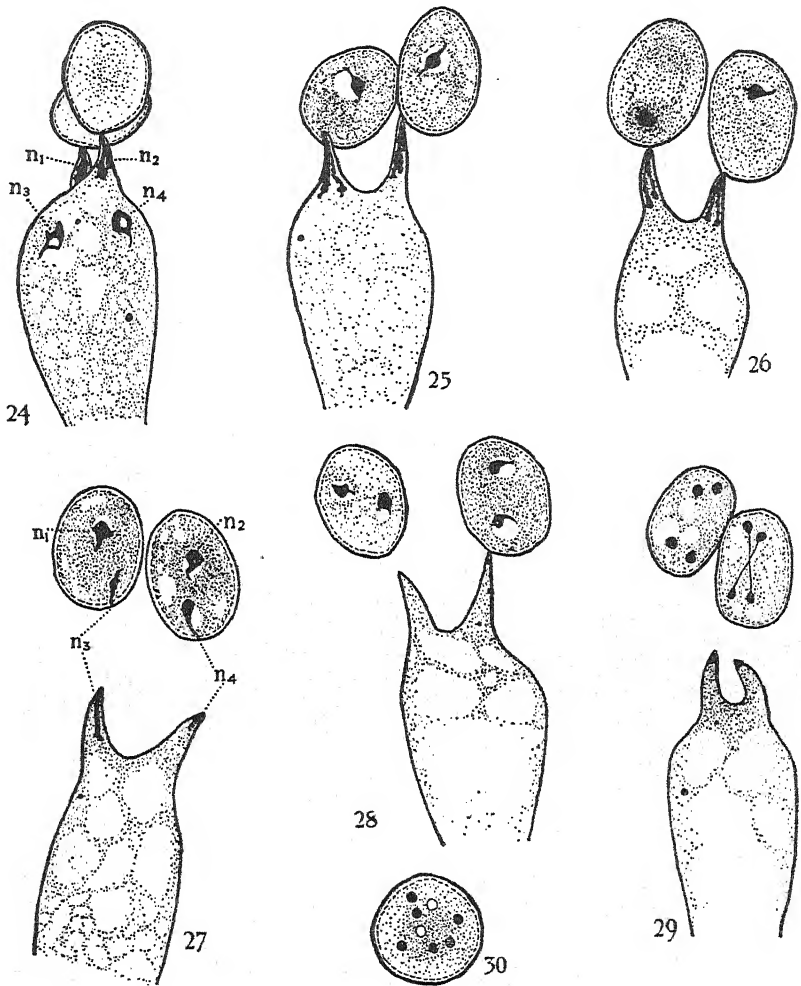
To summarize the facts, each spore of the two-spored variety receives two of the four basidial nuclei, each of which undergoes at least one division within the spore, and there are no degenerating nuclei left in the basidium.

SPORE FORMATION IN THE FOUR-SPORED VARIETY OF *P. CAMPESTRIS*.

The basidium in the four-spored variety of *P. campestris* produces four sterigmata (Fig. 34) each of which bears a spore (Fig. 35). The spore is at first thin-walled and colourless (Figs. 36 and 38) but it, like those of the two-spored variety, acquires a thick wall before it receives a nucleus (Fig. 37). The difficulties of manipulation and observation are greater in the case of a four-spored basidium than for a two-spored one for obvious reasons. It is only by great good fortune that all four spores remain attached to their sterigmata and in their normal relation to one another throughout the processes of cutting and staining. In a large number of the basidia examined only two spores had remained in position.

As soon as the spore-wall has become thick the four basidial nuclei move up from the base of the basidium (Fig. 39) assuming the hairpin-like shape *en route*. In this manner each spore receives one of the four basidial nuclei (Fig. 40). In one basidium examined (Figs. 41 *a* and 41 *b*) all four spores although detached from their sterigmata, had remained in a group above the basidium to which they belonged. In two of the four spores the nucleus can be seen in the process of regaining its spherical shape (Fig. 41 *b*). The other two spores had been in the act of receiving their respective nuclei at the time of fixation. The subsequent movement of the spores had broken the nuclei across, so that part of a nucleus can be seen in each spore and part in each sterigma (Fig. 41 *a*). The two figures, 41 *a* and 41 *b*, are different views of the same basidium showing two spores in focus in each view. The nuclei, after their passage into the spores, become reorganized and then undergo division (Fig. 42). All the uncoloured nearly mature spores examined in this variety contained two nuclei. No doubt a more extensive search would reveal the presence of an occasional four-nucleate spore. Like those of the two-spored variety the spores become dark in colour when ripe.

Thus in both varieties of *P. campestris* all four basidial nuclei come into play in spore formation. Where four spores are formed each spore

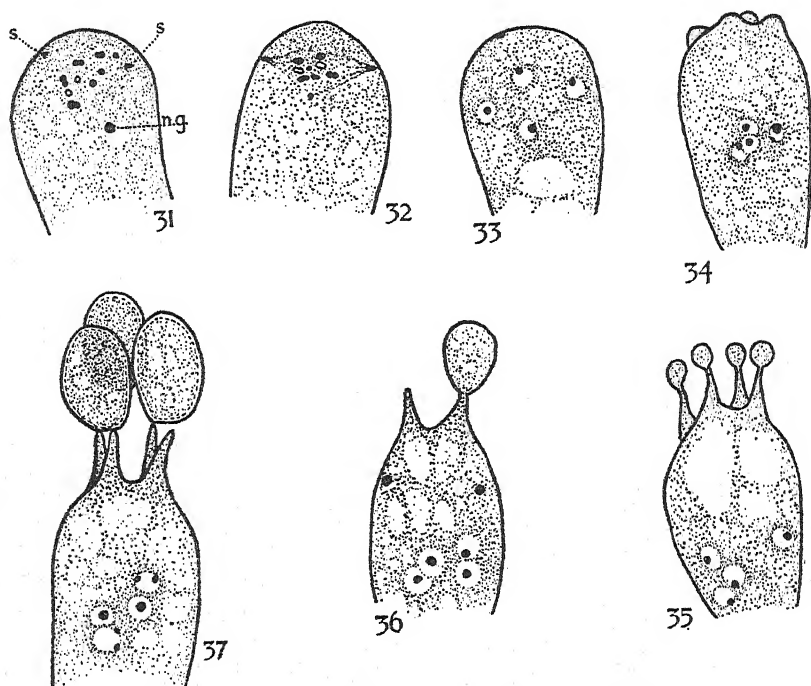


FIGS. 24-30. Two-spored form. 24. Nucleus in each sterigma, n_1 , n_2 , two nuclei still in basidium preparing to enter sterigmata, n_3 , n_4 . $\times 3,000$. 25. Nucleus in each spore and each sterigma. $\times 3,000$. 26. Same stage as previous figure. $\times 3,000$. 27. Spores artificially detached whole nucleus in each spore, n_1 , n_2 , part of a second nucleus in spore and part in sterigma n_3 , n_4 . $\times 3,000$. 28. Binucleate spores. $\times 3,000$. 29. Four-nucleate spores. $\times 3,000$. 30. Eight-nucleate spore. $\times 3,000$.

receives one basidial nucleus, and where only two spores are formed each spore receives two basidial nuclei. In both varieties these nuclei undergo at least one further division in the spore.

SPORE GERMINATION.

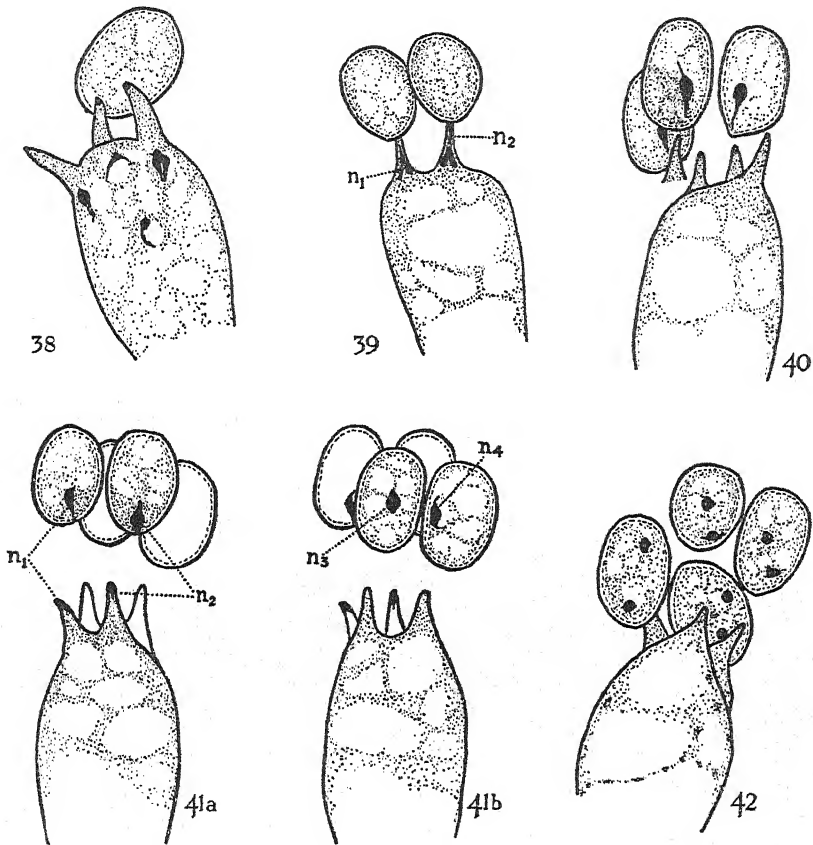
The extreme difficulty with which the spores of the mushroom germinate (22) is well known, and this difficulty has led to the substitution of tissue cultures for spore cultures in the preparation of the modern commercial product 'sterilized spawn' (7).



FIGS. 31-37. Four-spored form. 31. Late diakinesis with nine gemini. $\times 4,000$. 32. Metaphase of first division, nine gemini. $\times 4,000$. 33. Four-nucleate basidium. $\times 3,000$. 34. Young sterigmata. $\times 3,000$. 35. Young spores. $\times 3,000$. 36. Thin-walled spores, nuclei low in obliquely cut basidium. $\times 3,000$. 37. Thick-walled spores. $\times 3,000$. s = centrosome, n.g. = nucleolus.

In the present work the same difficulty has been encountered where single-spore cultures were used; none of the recognized methods employed induced separated spores to germinate. In several cases however, a mycelium was obtained when a very large quantity of spores was used as the inoculum. The veil of an unopened but nearly mature fructification was removed and the spores from it were allowed to fall on paper. Infections were made on to 3 per cent. malt agar, using large quantities of spores for each infection. In nearly all the cases several isolated centres of growth of a slowly growing mycelium were obtained after incubation for four days. The earliest stages of germination have not yet been observed, but confirmation as to its identity as the mycelium of *P. campestris* was obtained by comparison with the mycelium from tissue culture. The two mycelia

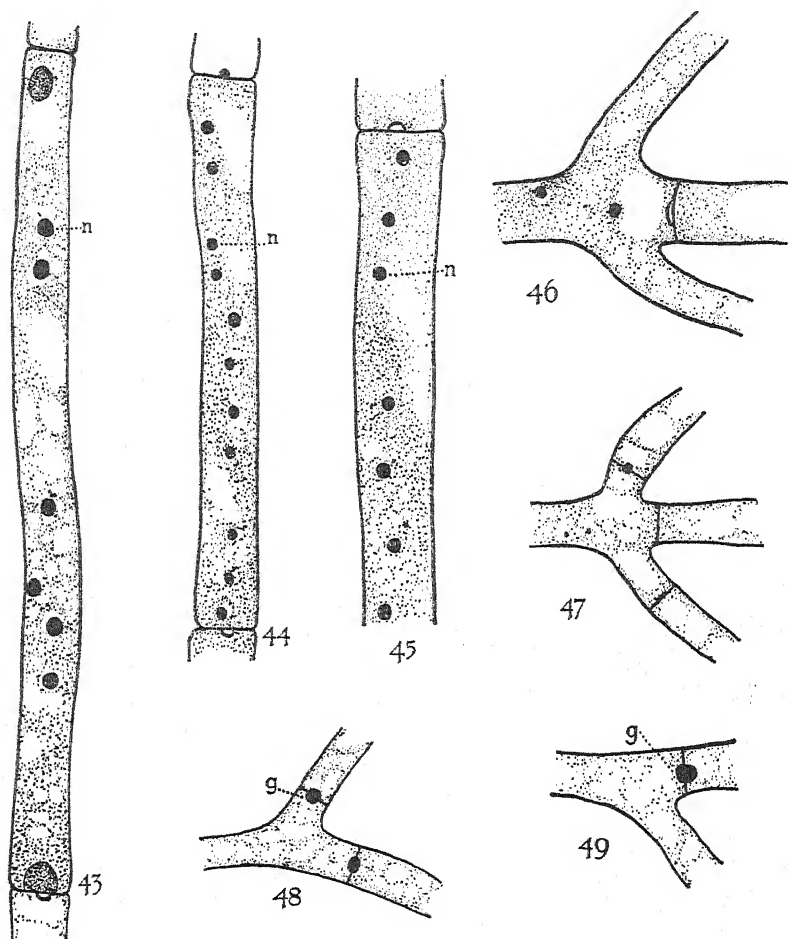
were compared carefully, using as many characters as possible and, as far as could be ascertained, were identical. The cells of both are long, narrow and multinucleate, each cell containing from six to ten nuclei (Figs. 43, 44,



FIGS. 38-42. Four-spored form. 38. Nuclei moving up to the sterigmata. $\times 3,000$. 39. Cut basidium with two of four spores and sterigmata, each sterigma containing nucleus. $\times 3,000$. 40. Uninucleate spores, basidium empty. $\times 3,000$. 41. *a* and *b*, two views of same basidium, n_1 and n_2 broken nuclei, part in spore and part in sterigma, n_3 and n_4 nuclei in spores. $\times 3,000$. 42. Binucleate spores. $\times 3,000$.

45). Lying against the cross-walls of the cells in both mycelia stainable granules can occasionally be seen, like those which are such conspicuous objects in the cells of the fruit body (Figs. 48, 49). The branching of the hyphae in both mycelia is often 'tri-radiate', (Figs. 46, 47) that is, three branches arise from the swollen distal end of one cell. In old cultures of mycelia from both spores and tissue cultures the individual hyphae show a marked tendency to become woven into complicated thick white strands. Taking all these similarities into account it seems reasonable to assume that the mycelium obtained from the infections with mushroom spores was

really that of *P. campestris* and not a stray infection. Therefore, although the earliest stages of germination have not been seen, it seems likely that



FIGS. 43-49. Mycelium from spores and tissue cultures. 43. Six-nucleate cell from spore mycelium. $\times 2,100$. 44. Cell with eleven nuclei from tissue culture mycelium. $\times 1,750$. 45. Portion of same cell. $\times 2,100$. 46. Branching of mycelium from spore. $\times 1,750$. 47. Branching of mycelium of tissue culture. $\times 960$. 48. Mycelium from spore with granule at septum. $\times 1,050$. 49. Mycelium from tissue culture showing granule. $\times 960$. n = nucleus, g = granule.

the cells of *P. campestris* are multinucleate from the germ-tube to the beginning of gill formation.

DISCUSSION.

From the study of the cytology of *Psalliota campestris* several points of interest arise for discussion. These will be taken in turn, beginning with

three minor questions for which, at the moment, no conclusions can be reached.

Firstly, the occurrence of initially uninucleate and initially binucleate spores, within one species, immediately raises questions as to the presence of heterothallism and homothallism in the four-spored and two-spored varieties respectively. Since, however, the difficulty of obtaining single spore cultures seems at the moment to be insuperable this side of the question must remain in the realms of speculation. Secondly, there is the origin and genetical nature of the two nuclei in the young basidium. If the binucleate condition is attained by a gradual reduction in the number of nuclei in successive cells of a branch, each hyphae acting independently. the value of the ultimate fusion is open to question. Even if there were two strains involved, in the absence of any other controlling factor, there would be at the most but a fifty per cent. chance that any pair of fusing nuclei would be of a different constitution. Again, no conclusion can be drawn until single-spore cultures have been obtained. Thirdly, only negative evidence has been forthcoming with regard to the suggestion that the centrosomes are responsible for the initiation of the sterigmata and the passage of the nuclei into the spores. Such a mechanism has not been seen in either variety of *P. campestris*, even when fixative and stain, said to demonstrate their presence, were employed.

The chromosome number ($n = 9$) established for *P. campestris* is a point worthy of mention. Although the actual number is relatively unimportant, reference to the literature shows it to be considerably higher than those (2-6) previously reported for Basidiomycetes (20) (34) (35). The number for *P. campestris* was arrived at by the study of more than one phase of nuclear division. Early in the work it was seen that counts made only at the anaphase of nuclear division in the basidium were unreliable. This unreliability is due to the tendency of the chromosomes to become massed together as they approach the poles of the spindle so that individual chromosomes are indistinguishable. Many of the very low numbers quoted for Basidiomycetes hitherto are probably due to counts being made with no reference to the appearance of the nucleus at diakinesis.

From the accounts of spore formation given above it may be seen that the development of the four-spored variety of *P. campestris* agrees well with the general description for the majority of four-spored Basidiomycetes. The peculiar hairpin-like shape already described, assumed by the nucleus during its passage into the spore (Fig. 40), is characteristic and unmistakable and is taken on by all the basidial nuclei in both varieties of mushroom. The nuclei are therefore easily recognized during transit through the sterigmata. In the two-spored variety the appearance of the second pair of nuclei, just before their journey through the sterigmata, when they are in the process of attaining the hairpin-like shape (Fig. 24)

has no doubt led to the statement that they degenerate within the basidium. Examination of a slightly later stage, however, (Fig. 25) reveals them passing through the sterigmata into the spores, proving conclusively that in *P. campestris* the second pair of nuclei follow the first pair into the spores and do not remain behind to degenerate in the basidium. While no general conclusions with regard to two-spored Basidiomycetes as a whole are either suggested or justified, what remains a positive statement of fact is, that the two-spored variety of *P. campestris* possesses initially binucleate spores. In this respect the development of *P. campestris* is comparable to that described for *Craterellus cornucopioides* (25) and *Hydnangium carneum* (26).

Lastly, there remains the question of the interrelationship of the two forms. Although collections of wild material were made from widely separated localities¹ in England, all these collections proved to be of the four-spored type. While more careful search would no doubt reveal the occurrence of undoubted wild (not escaped) two-spored *P. campestris*, it is obvious that the two-spored form, though common in cultivation, is far less frequently found in the field. This also appears to be the position in America (1). The knowledge that the cytology of the four-spored variety conforms to what is known for four-spored Basidiomycetes in general, together with the facts regarding its distribution, lead one to the conclusion that the two-spored variety of *P. campestris* can be regarded as the newer and derived form. This newer form has for some reason been the source of the cultivated mushroom.

SUMMARY.

1. *P. campestris* is known to exist in two varieties, the common wild form having four spores, the modern cultivated two.

2. All the cells of *P. campestris* from the young mycelium to the cells of the gills are multinucleate, the number of nuclei per cell diminishing as the gills are approached.

3. The cells of the trama are often binucleate, those of the subhymenial layer and of the hymenium are always binucleate.

4. Nuclear fusion takes place in the young basidium and meiosis follows immediately after fusion.

5. The haploid number of chromosomes for both forms of *P. campestris* is nine.

6. A second division follows meiosis in the basidium, so that the number of nuclei prior to spore formation is four.

7. In the *four-spored variety each spore receives one* of the four basidial

¹ Kent 1 locality, Sussex 2 localities, Hampshire 2 localities, Wiltshire 1 locality, Lancashire 1 locality.

nuclei. In the *two-spored variety* each spore receives one and then a second basidial nucleus. The shape of the nuclei during their passage into the spores is remarkable and unmistakable.

8. The nuclei of both varieties undergo at least one division within the spore.

9. The number of spores capable of germinating in culture is very small. The cells of the mycelium from spores and tissue cultures are multinucleate.

10. The question of spore germination, the possible presence of different mycelial strains and the ancestry of fusing nuclei are left aside for further work.

11. *P. campestris* is shown to have a higher chromosome number than any previously reported for the Hymenomycetes.

12. A comparison of the two forms of *P. campestris*, taking into account both the known facts as to their cytology and of their distribution, suggests that the two-spored form is probably the newer.

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Studies in the Genera *Cytosporina*, *Phomopsis* and *Diaporthe*.

VII. Chemical Factors Influencing Sporing Characters.

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With eight Figures in the Text.

CONTENTS.

	PAGE
I. INTRODUCTION	19
II. THE MATERIAL USED	20
III. THE EXPERIMENTAL METHOD	20
IV. GENERAL OBSERVATIONS ON SPORING ORGANS AND SPORE DEVELOPMENT	21
V. NITROGEN	23
VI. CARBOHYDRATE	24
VII. NITROGEN AND CARBOHYDRATE	30
VIII. ACIDITY, ALKALINITY	34
IX. SUBSIDIARY EXPERIMENTS	34
X. DISCUSSION	36
XI. SUMMARY	39

I. INTRODUCTION.

DAS GUPTA (2) observed that the sporing characters of certain saltant strains of *Cytosporina ludibunda* varied with kind of nutrient medium. Thus, in the case of strain C, when Richards' medium was used, 'B' spores were formed exclusively; on the other hand, in Coon's medium intergradation was encountered, that is to say, the spores ranged from short, straight 'A' spores through intermediate 'C' spores to long, bent 'B' spores. Das Gupta suggested that the variation from medium to medium indicated some influence of nutrient conditions on sporing characters, but the factors inducing such changes were not investigated. The present

¹ Owing to the death of the author in 1931, this paper has been revised by Dr. A. S. Horne.

author has endeavoured to obtain more exact information on this subject, using the method of cultural analysis adopted by Seth (12) and applying statistical methods when examining data. The results clearly show that variation in sporing characters is mainly conditioned by nitrogen and carbohydrate. The importance of carbohydrate and nitrogen as factors governing variation in sporing characters was previously established by Brown and Horne (1), and Horne and Mitter (8) as a result of studies in the genus *Fusarium*.

II. THE MATERIAL USED.

It was originally intended to use the strains CA₃, C, CA, and CC, specially mentioned by Das Gupta as showing intergradation and variation in sporing characters from one medium to another. Unfortunately C, CA, and CC had changed with the passage of time. They either failed to sporulate or produced very few sporing organs in the first subcultures made, and were therefore unsuitable. The scope of the work was necessarily widened to include allied species in the hope that one or more would show intergradation. A list of the available strains is given below :

1. *Cytosporina ludibunda* CA₃.
2. " " CA₃₋₁ saltant from CA₃.
3. *Phomopsis coneglanensis* Trav. Centraalbureau voor Schimmel-cultures, Baarn.
4. " " P₁₁ saltant from *P. coneglanensis*.
5. *P. citri*, Jaffa 1 (J'1)
6. " " Brazil 10 (B 10) } Low Temperature Station, Cambridge.
7. " " " 11 (B 11) }
8. *Phomopsis* sp. Rose stem (R) } Washington.
9. *Diaporthe*, no. 159 }

The author is greatly indebted to Mr. Tomkins for the cultures of *Phomopsis citri* and to Miss Jenkins for the species from Washington.

III. THE EXPERIMENTAL METHOD.

The composition of the medium used as a standard is as follows: asparagin, 2 gm.; glucose, 2 gm.; magnesium sulphate, 0.75 gm.; potassium phosphate, 1.25 gm.; agar, 15 gm. and water, 1,000 c.c. When preparing series of modified media the method adopted by Seth was closely followed. Modifications were made by varying concentration of asparagin (A) and glucose (G), by substituting sucrose (S) or fructose (F) for glucose, and by introducing and varying concentration of malic acid (Ac) or sodium carbonate (Al). Series of plate cultures were invariably prepared in duplicate and, unless stated otherwise, were kept at laboratory temperature.

The following methods were adopted to assist in obtaining reliable estimates of relative proportions of spores of different kinds and of spore dimensions :

Proportions. Spore suspensions were made, in 0.5 c.c. water, by crushing five sporing organs (pycnidia or stromata) obtained from a given zone of mycelium. Suitable microscopical preparations were made and percentage values calculated from numbers of 'A', 'B' or 'C' spores observed in five microscopical fields.

Dimensions. Measurements of length and breadth of fifty spores were made and the mean value recorded in each case. With the bent 'B' spores an attempt was made to measure the actual length.

Abnormal spores are described as shrivelled, vacuolated, atrophied, and so on, and are illustrated by camera lucida drawings. The drawings of spores found in given microscopical fields represent, as far as possible, the proportions in which different kinds of spores are actually present and, since the drawings are always made to the same scale, both change in length and change in proportions associated with altered composition of medium can be followed.

The test for homogeneity ((3), p. 196) was applied in order to determine whether estimates of percentages of spores and of spore dimensions made in the way described above are reliable. For testing length five microscopical preparations were made from the same suspension of spores, each preparation contributing fifty measurements, and for testing percentages, seven microscopical preparations were used, calculating percentage number of 'A' spores for each preparation from observations made in five microscopical fields. The following results were obtained:

Length 'A' spore	$Z = 0.345$	1 % point	0.5999
" 'B' "	$Z = 0.490$	5 % "	0.4319
Percentage 'A' "	$Z = 0.517$	1 % "	0.6303
		5 % "	0.4471

In two instances, viz. length of 'B' spores and percentage of 'A' spores, the values of Z exceed the values of the requisite 5 per cent. points, indicating that the populations are not homogeneous. The observed heterogeneity possibly reflects variation from one sporing organ to another of those which contribute to the suspension of spores. No significance is attached to differences recorded in the following pages where the values of Z calculated do not exceed the requisite 1 per cent. probability values.

IV. GENERAL OBSERVATIONS ON SPORING ORGANS AND SPORE DEVELOPMENT.

The sporing organs, in standard medium, vary from simple pycnidia to more or less irregular stromata. These organs decrease numerically and in size with increasing or decreasing concentration of sugar or nitrogen.

The time necessary for the development and maturation of the sporing organs varies with fungal strain and experimental conditions. In general,

strains which produce well-marked stromata, such as *Phomopsis coneglanensis*, *P. citri*, J and B 10, &c, need a longer time for maturation than strains which produce small stromata or pycnidia. Maturation is retarded by relatively high concentrations of glucose or asparagin, and with strains producing large stromata maturation may be delayed for several weeks.

The strains vary in the order in which the sporing organs develop in media. Thus, with *P. coneglanensis*, development and maturation proceed from the peripheral region inwards towards the centre of the culture, but in the case of *Cytosporina ludibunda*, CA₃, this order is reversed, the first sporing organs are observed near the centre of the culture and sporing spreads outwards.

Early experiments with *P. coneglanensis* and *C. ludibunda*, CA₃, gave irregular results. The irregularity was traced to saltation which caused obvious sectoring in certain members of asparagin and glucose series. Eventually a saltant of *P. coneglanensis* was isolated, which, unlike the parent, produced both 'A' and 'B' spores, and a saltant of *C. ludibunda*, CA₃, which, also unlike the parent, produced 'A' spores exclusively. The experimental work was then repeated, dealing separately with saltants and parents.

With certain strains, for example *C. ludibunda* CA₃, the proportions of 'A' and 'B' spores change with increasing age of the sporing organ. In this instance the first spores discharged are nearly all of 'B' type; as time goes on 'A' spores increase and 'B' spores correspondingly decrease numerically. Periodical records made from spores taken 1 cm. from the centre of a culture showed a gradual decrease in number of 'B' spores from 91.2 per cent., when the culture was twenty days old, to 70 per cent. after forty-one days. Spores taken from a position 3 cm. distant from the centre gave 'B' spores 97.7 per cent. after twenty-seven days and 59.9 per cent. after forty-eight days. The delayed production of 'A' spores at 3 cm. from the centre is due to the fact that development of sporing organs proceeds from the centre outwards, as already mentioned. In order to minimize errors introduced through variations of the kind mentioned above observations were limited to spores developing a given distance from the centre of the culture. Records of spore dimensions and proportions were made a few days after the first spores had emerged from the sporing organ.

The salient sporing characters shown by the strains under investigation in standard medium are as follows:

C. ludibunda, CA₃. Pycnidial development proceeds from the centre. Pycnidia embedded in thick tufts of mycelium, in zones. 'A' spores, 40 per cent., 'B' spores, 60 per cent., approximately.

C. ludibunda, CA₃₋₁. Pycnidial development proceeds from the centre. 'B' spores absent.

P. coneglanensis. Earliest stromata produced towards the periphery. 'B' spores absent.

P. coneglanensis, P. 1. Pycnidial development proceeds from the centre, pycnidia in thick tufts of mycelium. 'A' spores, 53 per cent., 'B' spores 47 per cent. approximately.

P. citri, J. 1. Earliest stromata formed at the centre, pycnidia in large black stromata. 'A' spores, 51 per cent., 'B' spores, 34 per cent., 'C' spores, 15 per cent. approximately.

P. citri, B 10, 11. Stromata scattered. 'B' spores absent.

V. NITROGEN.

(1) Strains which produce 'A' spores alone in standard medium: *P. coneglanensis*, *C. ludibunda*, CA₃₋₁.

Six concentrations of asparagin (1/3, 1, 3, 6, 9, and 12 A) were used in combination with 5 G.

No 'B' spores were produced throughout the series. At 1 A the 'A' spores were quite normal, but at 3 A they were relatively large and presented a vacuolated appearance. With further increase in asparagin the spores became more and more atrophied (Fig. 1).

The mean values obtained for length and breadth, using fifty spores, are given in Table I.

TABLE I.

Mean Values of Length and Breadth in μ of 'A' Spores.

Asparagin gm. per litre.	<i>P. coneglanensis</i> .		<i>C. ludibunda</i> , CA ₃₋₁ .	
	Length.	Breadth.	Length.	Breadth.
1/3	6.54	2.37	5.59	2.22
1	6.57	2.37	5.88	2.52
3	6.31	2.76	6.03	3.21
6	6.16	2.70	5.68	2.02
9	5.89	2.07	5.26	1.96
12	5.86	1.92	4.81	1.81

Analysis of variance of the data given in Table I yielded the following results:

<i>P. coneglanensis</i> ,	length $Z = 1.405$.	Differences of means	± 0.106 .
	breadth $Z = 1.593$.	" " "	± 0.095 .
<i>C. ludibunda</i> , CA ₁₋₃ ,	length $Z = 1.565$.	" " "	± 0.131 .
	breadth $Z = 2.049$.		± 0.094 .

All the values of Z greatly exceed 0.5522, the 1 per cent. probability value ((3) Table VI) indicating that treatment has been effective in modifying spore dimensions. The principal effect is seen in the case of abnormal

spores. Thus at 3 A, where vacuolation was observed, the spores reach maximal size. With concentration rising from 3 A to 12 A size decrease is associated with increasing atrophy, the atrophied spores formed at 12 A being very much smaller than normal spores. The differences given by $1/3$ A v 1 A for length (0.29 ± 0.131) and breadth (0.30 ± 0.094) of normal spores of *C. ludibunda* CA₃₋₁ are significant.

(2) Strains which produce 'A' and 'B' spores in standard medium. *C. ludibunda*, CA₃.

Four concentrations of asparagin (1, 2, 4, and 8 A) were used in combination with 8 G.

The principal effects observed with increasing concentration are as follows: (a) Percentage number of 'B' spores decreases, the values recorded being, 1 A, 47.7; 2 A, 41.0; 4 A, 34.0; 8 A, 0.0. (b) Average length of 'B' spores decreases from $22.7 \pm 0.40 \mu$ (1 A) to $14.6 \pm 0.40 \mu$ (4 A). (c) 'A' spores diminish in size and become atrophied, as recorded above for the saltant derived from this parent.

VI. CARBOHYDRATE.

(1) Strains which produce 'A' spores alone in standard medium. (a). Relatively low concentrations of glucose.

Five concentrations of glucose (1, 3, 6, 9, and 12 G) combined with 2 A were used. The strains tested were *C. ludibunda*, CA₃₋₁ and *P. coneglanensis*.

'A' spores alone were produced throughout the series. At 1 G and 3 G the spores observed were irregular and vacuolated; at higher concentrations the spores were normal. The following estimates of mean length and breadth of spores of *C. ludibunda*, CA₃₋₁ were obtained: Length 1 G, 5.82 ± 0.058 ; 3 G, 5.91 ± 0.063 ; 6 G, 5.98 ± 0.058 ; 9 G, 6.00 ± 0.066 ; 12 G, $6.10 \pm 0.070 \mu$. Breadth, 1 G, 2.56 ± 0.056 ; 3 G, 2.83 ± 0.061 ; 6 G, 2.59 ± 0.060 ; 9 G, 2.61 ± 0.061 ; 12 G, $2.66 \pm 0.062 \mu$. There is a distinct tendency for length to increase with increasing glucose, perhaps conditioned by the presence of abnormal spores at 1 G and 3 G. The estimates of mean breadth are irregular.

(b). Relatively high concentrations of glucose.

Four concentrations of glucose (16, 32, 64, and 128 G) combined with 2 A were used. The strains tested were *P. coneglanensis*, *P. citri*, B 10 and B 11, and *Diaporthe*, 159. The object of this experiment was to determine whether 'B' spores would be produced at higher concentrations of glucose than those used in the previous experiment.

Diaporthe, 159, alone failed to produce 'B' spores. With the remaining fungi, no 'B' spores were observed at concentrations below 128 G. The

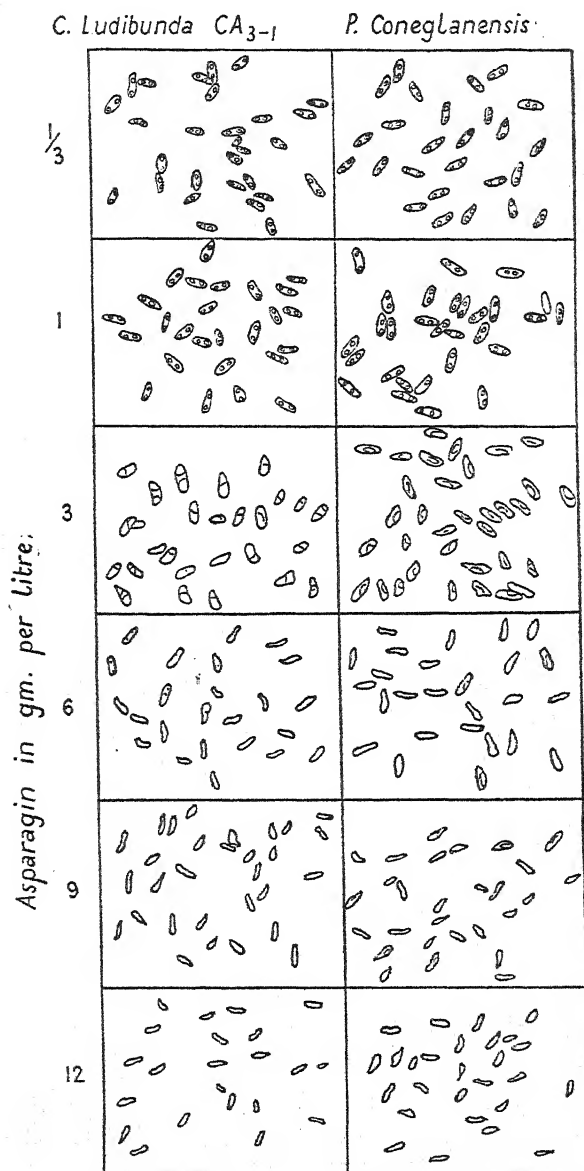


FIG. 1. Illustrates variation in 'A' spores conditioned by asparagin. *Phomopsis coneplanensis*, *Cytosporina ludibunda*, CA₃₋₁.

percentage values obtained at 128 G were: *P. coneglanensis*, 75, with mean length 25.3μ ; *P. citri*, B 10, 62.1, with mean length 32.6μ ; and *P. citri*, B 11, < 1. Spores of *P. citri*, B 10, as observed at 128 G are illustrated in Fig. 2.

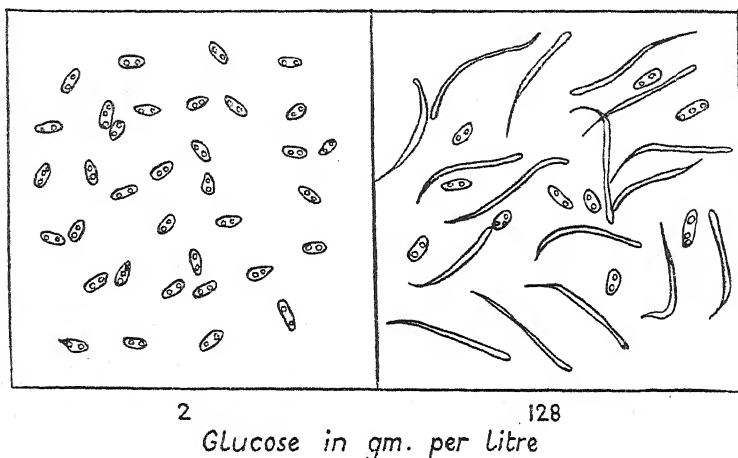


FIG. 2. Illustrates the effect of high concentration of glucose on the spores of *P. citri*, B 10.

(c). Cane sugar, maltose, and fructose.

Three series of media were prepared in which cane sugar, maltose and fructose were substituted for glucose, using four concentrations (4, 16, 64, and 128 C, M, and F) of each. The fungi used were *P. coneglanensis* and *C. ludibunda*, CA₃₋₁.

Again, no 'B' spores were observed at concentrations below 128 C, M, or F. The percentage number of 'B' spores recorded for 128 gm. of sugar per litre together with mean length, in μ , will be found in Table II.

TABLE II.

Production of 'B' Spores in Media containing 128 gm. of Sugar per Litre

Fungus.	Sugar.	Per cent.	Mean length in μ .
<i>P. coneglanensis</i>	sucrose	83.4	25.30 ± 0.40
	maltose	80.8	24.96 ± 0.44
	fructose	65.6	25.91 ± 0.41
<i>C. ludibunda</i> , CA ₃₋₁	sucrose	85.4	28.58 ± 0.56
	maltose	96.7	28.96 ± 0.62
	fructose	98.1	28.09 ± 0.45

It is seen from Table II that kind of sugar has little effect on spore length, but there is a marked effect on percentage number of 'B' spores. Thus, with *P. coneglanensis* the percentage varies from 65.6 (fructose) to

83.4 (sucrose). The results also vary with fungal strain. With *C. ludibunda*, CA₃₋₁, the highest percentage is given by fructose; with *P. coneglanensis*, on the other hand, the lowest percentage is recorded for this

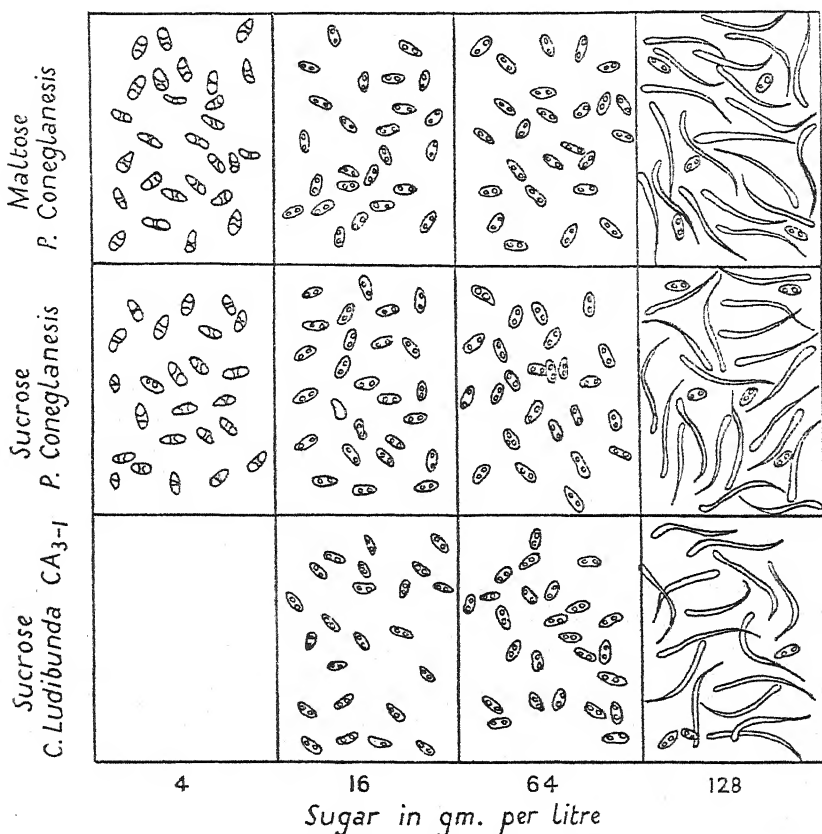


FIG. 3. Illustrates variation in sporing characters conditioned by sugar, *P. coneglanensis*, *C. ludibunda*, CA₃₋₁.

sugar. The two fungi also differ as regards length of 'B' spores. The results of this experiment are illustrated by the drawings of spores given in Fig. 3.

(2) Strains which produce 'A' and 'B' spores in standard medium. *C. ludibunda*, CA₃.

Series of media were prepared to contain 4, 8, 16, 32, and 64 gm. of glucose per litre.

The percentage values and mean length in μ calculated for 'B' spores, based on fifty spores in each case, are as follows: percentage number, 4 G, 16.9; 8 G, 41.0; 16 G, 44.1; 32 G, 50.1; 64 G, 97.2; length, 4 G, 15.3; 8 G, 18.0; 16 G, 21.9; 32 G, 22.8; 64 G, 24.2.

Analysis of variance of spore length gave $Z = 2.1727$, a value which greatly exceeds 0.7443, the requisite 1 per cent. probability value, indicating that concentration of glucose has proved very effective in modifying length of 'B' spores. The results showed that 'B' spores gradually replace 'A' spores and at the same time longer spores are produced with increasing glucose.

P. coneglanensis, 1.

Series of media were prepared to contain 8, 16, 32, and 64 gm. of glucose per litre.

Percentage number of 'B' spores recorded together with mean length, in μ , of 'A' spores and of 'B' spores, based on fifty spores in each case, are as follows: Percentage number, 8 G, 44.8; 16 G, 58.6; 32 G, 91.5; 64 G, 97.9; length of 'A' spores, 8 G, 5.94; 16 G, 6.03; 32 G, 6.13; 64 G, 6.37; length of 'B' spores, 8 G, 20.46; 16 G, 23.30; 32 G, 24.24; 64 G, 25.44.

Analysis of variance of spore length gave in the case of 'A' spores, $Z = 1.049$ and, in the case of 'B' spores, $Z = 1.820$; with differences of means ± 0.093 and ± 0.488 respectively. The requisite 1 per cent. probability value (0.6651) is again greatly exceeded. The results show general agreement with those recorded above.

Phomopsis, R.

Media were prepared to contain 16 and 96 gm. of glucose per litre.

Percentage number and mean length, in μ , of 'B' spores recorded were: Percentage, 16 G, 21; 96 G, 98.4; length, 16 G, 22.7 ± 0.37 ; 96 G, 27.24 ± 0.45 . Once more agreement with the foregoing results is evident.

(3) Strains which produce 'A', 'B', and 'C' spores in standard medium. *P. citri*, J 1.

Series of media were prepared to contain 8, 16, 32, and 64 gm. of glucose per litre.

Percentage values together with mean length, in μ , of 'A', 'B', and 'C' spores are given in Table III.

TABLE III.

P. citri, J 1. Spore Variation in Relation to Glucose Concentration.

Glucose in gm. per litre.	Percentage			Mean length in μ .		
	'A'.	'B'.	'C'.	'A'.	'B'.	'C'.
8	97.0	0.0	3.0	6.16	—	8.92
16	48.4	43.7	7.9	6.06	23.58	10.47
32	40.0	53.8	6.2	6.19	24.12	11.73
64	7.0	82.9	10.1	6.52	26.82	12.39

Analysis of variance of length of 'A', 'B', and 'C' spores, using in each case complete sets of data, gave:

'A' spores $Z = 0.975 (0.7636)$; differences of means ± 0.108 .
 'B' " $Z = 1.686 (0.6651)$; " " " ± 0.454 .
 'C' " $Z = 1.942 (0.6651)$; " " " ± 0.310 .

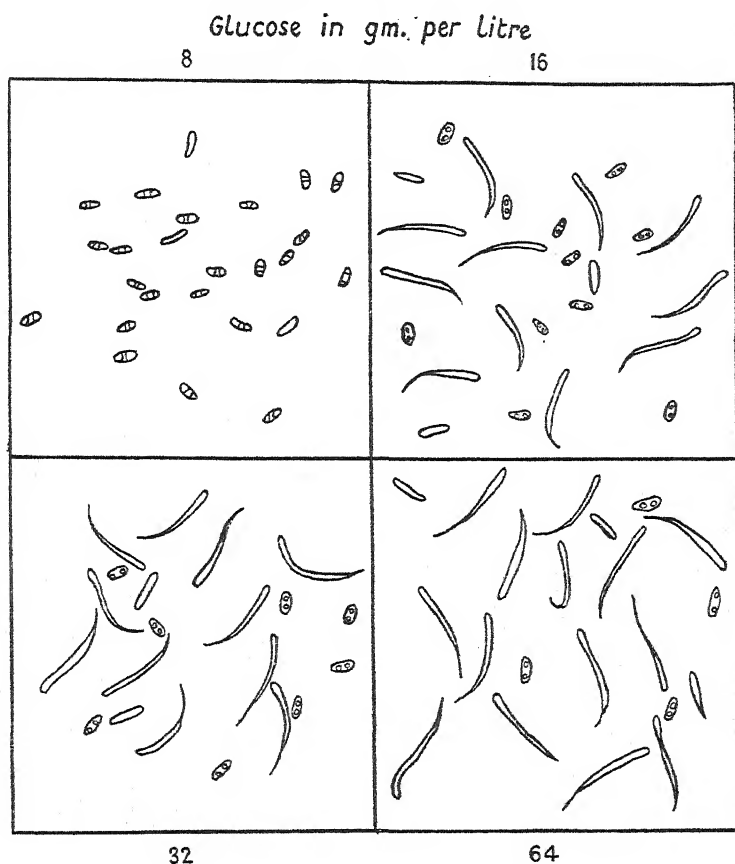


FIG. 4. Illustrates variation in sporing characters conditioned by glucose. *P. citri*, J1.

All the values of Z exceed the requisite 1 per cent. probability values (bracketed), and therefore treatment has proved effective in modifying length irrespective of kind of spore. In each case length increases with increasing glucose. At the same time spores of 'A' type are replaced by spores of 'B' and 'C' types, 'B' spores greatly predominating. The results are illustrated by the drawings given in Fig. 4. There are two to three 'C' spores in each of the microscopical samples represented. No difficulty was experienced in distinguishing between 'A', 'B' and 'C' spores.

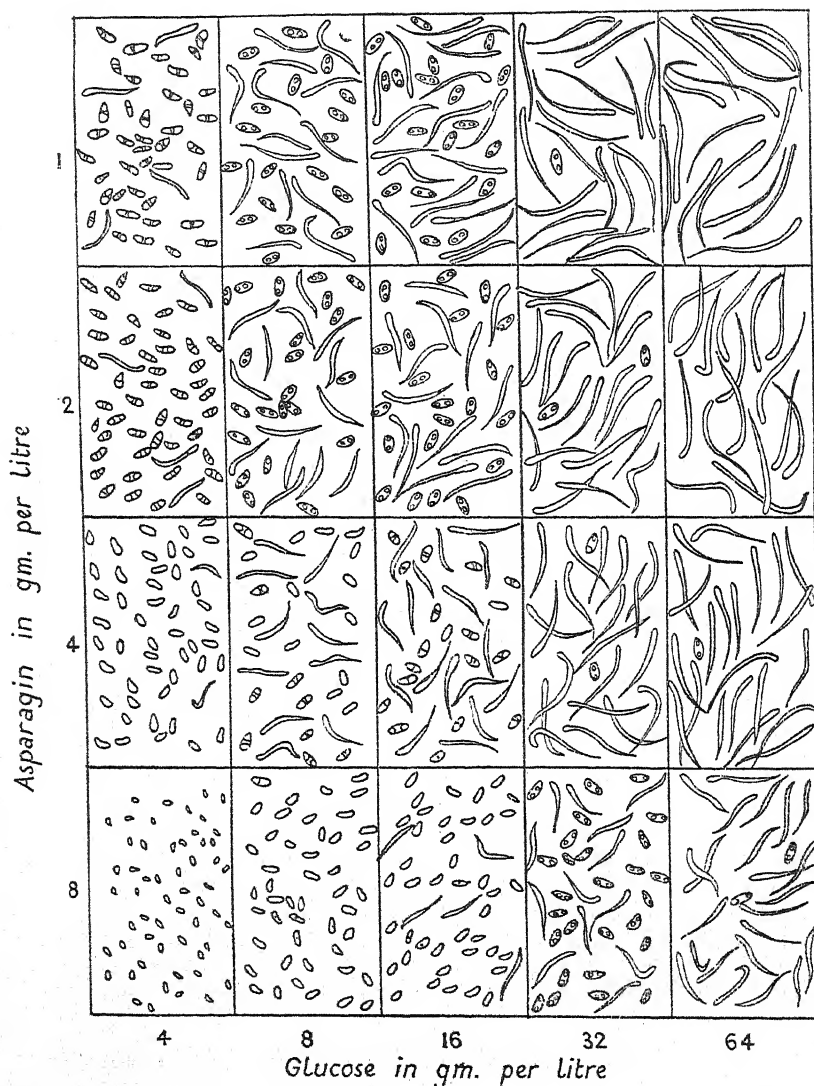


FIG. 5. Illustrates variation in sporing characters conditioned by glucose and asparagin.
C. ludibunda, CA_3 .

VII. NITROGEN AND CARBOHYDRATE.

(1) Strains which produce 'A' and 'B' spores in standard medium.

C. ludibunda, CA_3 .

Four concentrations of asparagin (1, 2, 4, and 8A) were used in combination with five concentrations of glucose (4, 8, 16, 32, and 64 G), making twenty combinations in all.

The general effect of treatment on shape, size, and relative proportions of 'A' and 'B' spores is illustrated by the drawings given in Fig. 5. It is seen that relatively high asparagin (4 A-8 A) has a marked effect on shape of 'A' spores at low concentrations of glucose; the spores are irregular and atrophied. With increasing glucose 'A' spores become less and less adversely affected by high asparagin and develop normally at high concentrations of glucose (32 G-64 G).

Percentage numbers of 'B' spores, based on observations in five microscopical fields in each case, are given in Table IV together with mean length in μ of 'B' spores, calculated as usual from fifty measurements of length.

TABLE IV.

C. ludibunda, CA₃. *Effect of Glucose and Asparagin on 'B' Spores.*

	Percentage number.				
	4 G.	8 G.	16 G.	32 G.	64 G.
1 A	13.0	48.0	49.3	97.3	100.0
2 A	16.9	41.0	44.1	90.1	97.2
4 A	7.2	34.0	46.4	92.1	94.6
8 A	0.0	0.0	12.1	34.2	90.0
	Length (μ).				
1 A	18.72	22.68	22.50	23.52	30.00
2 A	15.30	18.00	21.90	22.80	24.24
4 A	11.64	14.58	19.44	22.38	23.46
8 A	—	—	12.42	17.16	19.14

It will be seen from Table IV that both percentage and length increase with increasing glucose irrespective of asparagin concentration; with increasing asparagin, on the contrary, length decreases and production of 'B' spores is retarded. The depressing effect of asparagin on production is, however, gradually counteracted as shown by the high values recorded for 64 G. These results are represented graphically in Figs. 6 (percentage) and 7 (length).

Since there are only three asparagin treatments available for analysis of variance of spore length, there will be fifteen sets in all, each with fifty variates, giving 749 degrees of freedom, of which 735 will represent error. There are five treatments with glucose and three with asparagin, giving 4 and 2 degrees of freedom, respectively, leaving 8 for the interaction of glucose with asparagin. The results of the analysis are given in Table V.

The results corroborate those given by previous analyses where effects due to glucose and asparagin have been considered separately. In addition, the significant interaction (A v. G) indicates a real differential effect of one chemical constituent on another in modifying length of 'B' spores. This effect is reflected in the curves shown in Fig. 6 which change in form with

asparagin increasing from 1 to 8 gm. per litre. Turning to the percentage data, a similar differential effect is seen in the diminishing power of asparagin

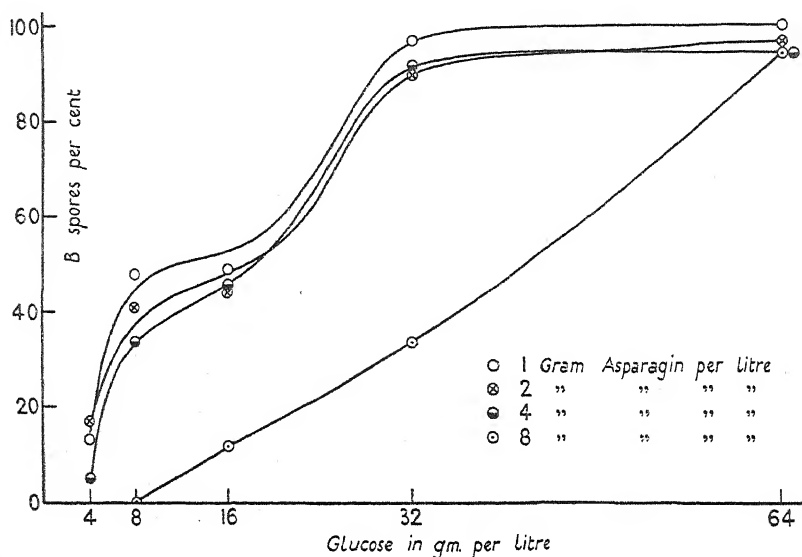


FIG. 6. Graph showing percentage number of 'B' spores in relation to concentration of glucose for four concentrations of asparagin. *C. ludibunda*, CA₈.

to check formation of 'B' spores as the concentration of glucose increases as already noted.

TABLE V.

Analysis of Variance. Length of 'B' Spores.

	D/F.	Variance.	S.D.	Log _e S.D.	Z.	1 % point.
Asparagin (A)	2	1696.944	41.19	3.7182	2.5965	0.7636
Glucose (G)	4	2532.330	50.32	3.9184	2.7967	0.5999
A v G	8	135.999	11.66	2.4562	1.3345	0.4604
Error	735	9.427	3.07	1.1217		

$$\text{Differences of means } \pm \frac{3.07}{\sqrt{n}} \times \sqrt{2}.$$

Phomopsis, R.

Media were prepared containing 96 G and 2 A, and 96 G and 10 A.

Percentage number and length, in μ , of 'B' spores recorded were: 2 A, 98.4 and 27.24 ± 0.45 ; 10 A, 29.8, and 20.34 ± 0.42 respectively. The percentage value for 10 A combined with 96 G falls a little below that given in Table IV (*C. ludibunda*, CA₃) for 8 A combined with 64 G.

(2) Strains which produce 'A' spores alone in standard medium.

Five concentrations of glucose (8, 16, 32, 64, and 128 G) were used in

combination with four concentrations of asparagin (2, 4, 8, and 16 A). *P. coneglanensis* was selected for the test.

As before (Section VI) 'B' spores were absent from media with concentration of glucose lower than 128 gm. per litre. At 128 G, the following

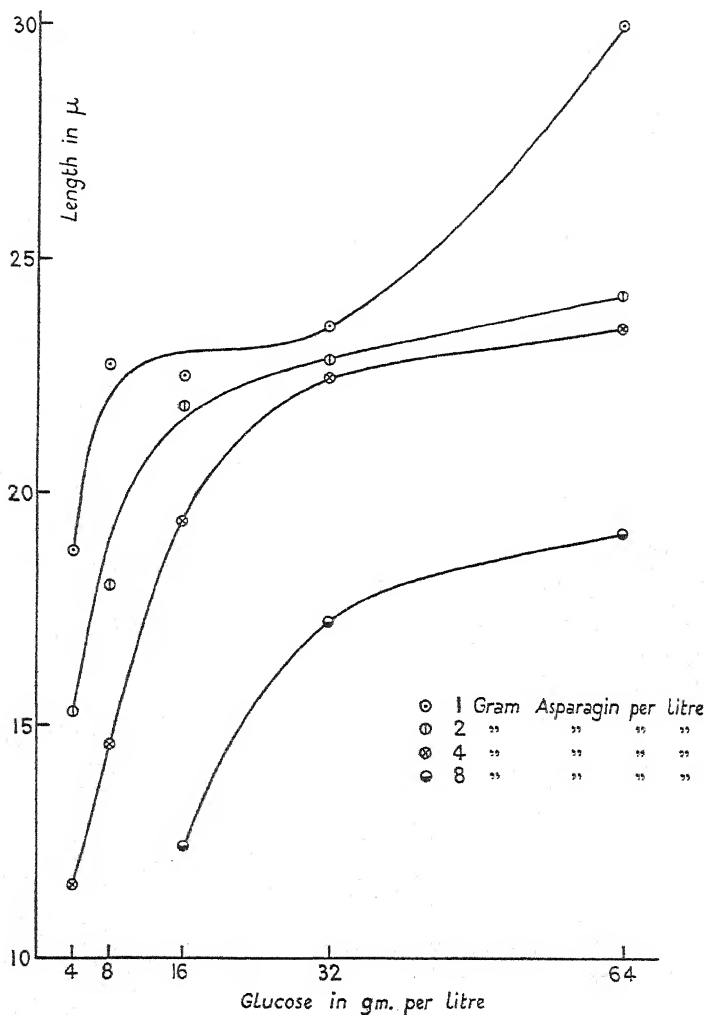


FIG. 7. Graph showing length of 'B' spores in relation to concentration of glucose for our concentrations of asparagin. *C. ludibunda*, CA₅.

estimates of percentage and mean length, in μ , of 'B' spores were obtained: 2 A, 72.5, 25.32 ± 0.32 ; 4 A, 72.4, 24.90 ± 0.40 ; 8 A, 27.7, 23.70 ± 0.39 ; 16 A, 6.1, 22.86 ± 0.35 . The data show that percentage falls and length diminishes with increasing asparagin (Fig. 8).

VIII. ACIDITY, ALKALINITY.

Series of media were prepared by adding malic acid or sodium carbonate, in proportions given in Table VI, to standard medium containing 1 per cent. starch. The fungus used was *P. coneglanensis*, 1, which, as already mentioned, gives both 'A' and 'B' spores in standard medium. In Table VI the average percentage number of 'B' spores observed under each treatment is given. The average is based, in each case, on estimates made for five microscopical fields.

TABLE VI.

P. coneglanensis, 1. *Effect of Acid or Alkali on Production of 'B' Spores.*

	pH.		% 'B' spores.
	Initial.	Final.	
0.0 Ac	7.4	8.4	41.6
0.5 Ac	4.9	8.6	45.2
1.0 Ac	4.5	8.6	45.8
2.0 Ac	4.2	8.5	44.8
4.0 Ac	3.9	8.4	41.4
8.0 Ac	3.1	8.2	43.4
16.0 Ac	2.8	7.6	46.0
0.5 Al	8.6	8.9	42.0
1.0 Al	8.8	9.1	42.4
2.0 Al	9.2	9.3	40.8

The range of variation shown in Table VI is very narrow and hence it would be unsafe to assume that treatment has had any real effect on proportions of 'A' and 'B' spores.

The effect of varied acid and alkali on length of 'A' and 'B' spores was also tested by comparing differences of mean values, calculated for the series specified in Table VI, with their requisite standard errors. The results showed that treatments had proved ineffective in modifying dimensions.

A second and similar experiment was made, using the parent strain ('A' spores only in standard medium). No 'B' spores were produced throughout the series. 'B' spores were produced in acid media solely when such media contained high glucose; the percentage estimated at 90 G, 135 G, and 170 G being 8.1, 57.5, and 76.4 respectively.

IX. SUBSIDIARY EXPERIMENTS.

Since the experimental work described in the preceding pages has been carried out at laboratory temperatures it was necessary to test the effect of temperature on length and proportions of 'A' and 'B' spores. Five sets of plates were prepared, using standard medium containing 1 per cent. starch, and kept at 15° C., 20° C., 25° C., 30° C. and laboratory

temperature, respectively. The fungi employed were *C. ludibunda*, CA₃₋₁ and the parent and saltant strains of *P. coneglanensis*.

The principal effect of temperature is seen in earlier development of

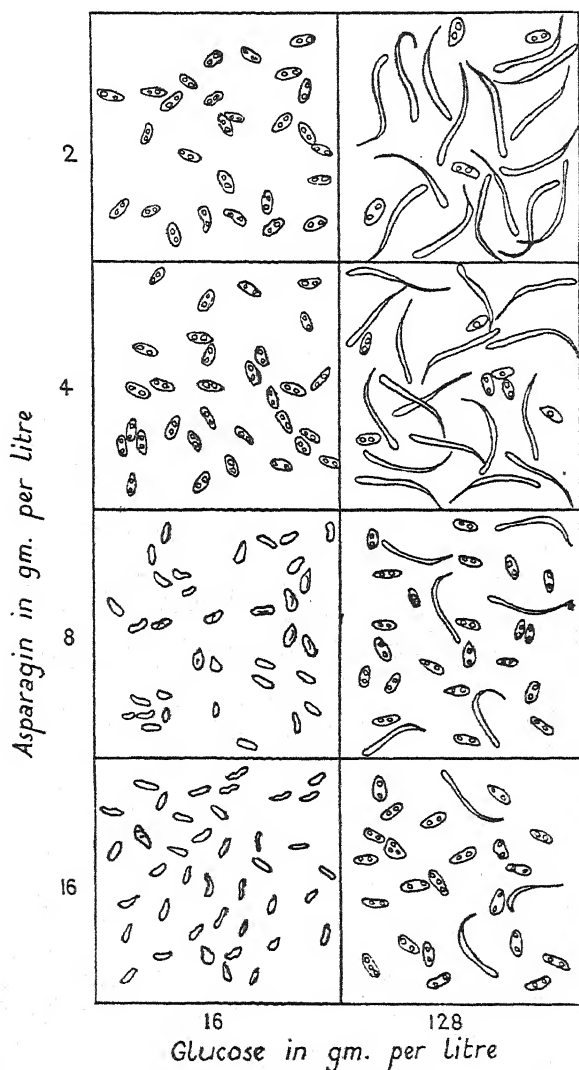


FIG. 8. Illustrates variation in sporing characters conditioned by glucose and asparagin. *P. coneglanensis*.

sporing organs at temperatures higher than 20°C. Relative proportions of 'A' and 'B' spores were not affected by varying the temperature. Mean length, in μ , of spores of *P. coneglanensis*, 1, calculated for each

temperature, together with differences of mean values (laboratory temperature *v.* constant temperature, in each case) and the requisite standard errors are given in Table VII. Mean values to which significance is attached are given in heavy type.

TABLE VII.

P. coneglanensis, 1. *Effect of Temperature on Spore Length.*

	'B'		'A'	
	Mean length.	Difference of means.	Mean length.	Difference of means.
15° C.	22.62 ± 0.33	0.66 ± 0.48	6.04 ± 0.074	0.27 ± 0.105
20° C.	23.88 ± 0.27	0.60 ± 0.45	6.10 ± 0.082	0.21 ± 0.110
25° C.	20.70 ± 0.32	2.58 ± 0.48	5.79 ± 0.063	0.52 ± 0.097
30° C.	19.80 ± 0.36	3.48 ± 0.51	5.76 ± 0.074	0.55 ± 0.105
Laboratory	23.28 ± 0.35		6.31 ± 0.073	

The results show that spore length diminishes with temperature increasing beyond 20° C. In the case of *P. coneglanensis* mean length of 'B' spores produced in standard medium containing 128 gm. of glucose per litre was similarly reduced; thus length was reduced from $25.32 \pm 0.32 \mu$ (laboratory temperature) to $20.64 \pm 0.41 \mu$ (30° C.), the difference of mean values being 4.68 ± 0.53 . It is clear from these results that error due to the fluctuations in temperature usually experienced in the laboratory is almost negligible.

The possibility that variable illumination may introduce error was also considered. Using various fungi, series of cultures were prepared and received the following treatments: (a) continuous artificial light emitted from an electric lamp provided with a water jacket to counteract heat generated by the lamp; (b) continuous darkness, and (c) variable illumination under the usual laboratory conditions. It was found that sporing was very prolific under continuous light; continuous darkness, on the other hand, did not favour development of sporing organs. Variations in proportion of 'A' and 'B' spores and in mean length of either kind, associated with light treatment, were unimportant.

X. DISCUSSION.

The results presented in the foregoing pages show conclusively that both sugar and asparagin are effective in modifying numerical proportions of the different kinds of spores and dimensions of spores produced by species and strains of the genera considered in this paper.

The effect of varying the sugar content of the standard medium on spore production was as follows: (1) With one exception, viz. *Diaporthe*, 159, all strains producing 'A' spores alone in standard medium gave 'B'

spores when concentration of sugar reached 128 gm. per litre; (2) with all strains producing both 'A' and 'B' spores in standard medium, percentage of 'B' spores increased with rising concentration until eventually all the spores produced were of 'B' type; (3) with *P. citri*, JI, which produces 'A', 'B', and 'C' spores in standard medium, numbers of 'B' and 'C' spores increased with rising concentration, but the former greatly predominated. Since complete intergradation was not observed in this instance the three kinds of spores may possibly be regarded as distinct types.

In every instance dimensions of spores tend to increase with rising concentration of sugar.

In contrast with sugar, an increase in the asparagin content of the standard medium does not in any circumstances induce formation of 'B' spores, and with strains producing spores of 'A' and 'B' type, 'B' spores decrease numerically with increasing concentration of asparagin. The tendency to check development of 'B' spores may be overcome by increasing the sugar content of media rich in asparagin.

Length of 'B' spores decreases with increasing asparagin. 'A' spores become vacuolated with concentration reaching 3 gm. per litre, and atrophied at higher concentrations.

In the case of *Fusarium*, according to Horne and Mitter (8) both high carbohydrate and high asparagin content of medium are associated with reduction in septation and atrophy of the spore. With *Phomopsis* and *Cytosporina*, atrophy is associated solely with high asparagin content. The effect of high glucose content is seen in replacement of fertile 'A' spores by 'B' spores, and these are generally regarded as infertile structures.

Several instances have been recorded where formation of 'B' spores in sporing organs found under natural conditions is confined to parts of the host plant which are rich in carbohydrate. Thus Roberts (11), referring to *P. citri* found on the Yellow Newtown apple, states: 'Pycnidia developed on the leaves show less tendency to form the long, curved spores than those growing on either fruit or branches.' Again, Harter and Field (7) observed that the stylospores ('B' spores) of *D. Batatatis* are commonly found on the roots and stems of the host (sweet potato) but have never been observed on the leaves or petioles. Pycnidia formed on the leaves develop only 'A' spores. Growths on corn meal or sweet potato agar derived from pycnidia obtained from leaves, however, produced both 'A' and 'B' spores. Harter and Field state that media which favour production of stylospores are rich in carbohydrate; stylospores were not produced in media rich in nitrogen.

In the case of *C. ludibunda*, CA₃₋₁, relative proportions of 'A' and 'B' spores vary with age of sporing organ; the first spores discharged are of 'A' type, 'B' spores forming later. Variation of this kind possibly explains the observation made by Harter (6) for *D. phaseolorum*, viz.

'specimens which practically bore no stylospores in the fall bore them in increasing numbers throughout the winter. As the season advances the stylospores increase in number and appear to be associated though not always with a saprophytic existence.' Here the change from one type of spore to another may proceed slowly at winter temperatures. The variation noted above is regarded as of genetic nature rather than as one primarily conditioned by nutrition because, owing to utilization of sugar by the fungus, concentration of sugar will fall with increasing age of fungus, and consequently older nutrient medium will not favour production of 'B' spores. Hahn (4) gives an instance (*P. conorum*) where, in cultures, 'B' spores were generally found associated with very few 'A' spores, *the latter developed later*.

In 1930, Hahn gave a comparative account of the species of *Phomopsis* occurring on Coniferae based on observations of sporing characters shown in material collected under natural conditions, on natural media (steamed twigs, &c.) and in nutrient media, viz. sugar corn meal (5), oat agar (10), and Leonian agar (9). Reference to Hahn's data shows that spore dimensions did not vary greatly from one nutrient medium to another. Thus with *P. occulta* the average length of 'A' spores in the three media mentioned above was 7.2 μ , 7.1 μ , and 7.1 μ respectively. Much wider variation was recorded from different hosts, for example, *Pseudotsuga Douglasii*, 7.4 μ ; *Thujaopsis dolobrata*, 6.6 μ ; *Sequoia gigantea*, 5.6 μ . Similar differences were recorded for steamed twigs, for example, *P. Douglasii*, 7.5 μ ; *Ulmus campestris*, 6.7 μ , &c. Results obtained by the present author suggest that the restricted range of variation found in the nutrient media is probably due to their relatively low carbohydrate content, while the variation from host to host possibly reflects differences in available carbohydrate, and, in the case of *T. dolobrata* and *S. gigantea*, lower carbohydrate. Hahn records both 'A' and 'B' spores for *P. occulta*, *P. Juniperovera*, *P. conorum*, and *P. montanensis*, 'B' spores occurring commonly in nature but with ratio of 'A' to 'B' extremely variable. This wide variation may be partly due to age of fungus and partly variation directly reflecting the chemical composition of host material.

Hahn separates the species of *Phomopsis* occurring on Coniferae into two groups based on the structure of the sporing organ and then subdivides the species according to the kind of spore produced. Under 'A' and 'B' spores, the four species mentioned above, and under 'A' spores, *P. strobis*, *P. Pseudotsuga*, *P. abietina*, and *P. Boycei* are given. A single strain of *P. occulta* is given under 'B' spores. The classification is founded mainly on sporing characters observed in media having low carbohydrate content. In this case, five species, or strains (*P. coneglanensis*, *C. ludibunda*, CA₃₋₁, *P. citri*, B 10 and B 11, and *Diaporthe*, 159) gave 'A' spores alone in a medium poor in carbohydrate (standard). But when concentration of

sugar was raised to 128 gm. per litre all the strains, with one exception (*Diaporthe*, 159), produced 'B' spores, as already mentioned. Hence it is doubtful whether the specific distinctions made by Hahn are valid.

XI. SUMMARY.

Variation in sporing characters observed by Das Gupta when various strains of *C. ludibunda* were grown in certain nutrient media (Coon's, Richards', &c.) has been investigated by the method of cultural analysis adopted by Seth. The results clearly show that such variation is mainly conditioned by nitrogen (asparagin) and carbohydrate.

With increasing concentration of sugar, the following results were obtained: (1) strains producing 'A' and 'B' spores in standard medium (glucose 2 gm. per litre), 'B' spores increase and 'A' spores correspondingly decrease numerically; at 128 gm. per litre all the spores are of 'B' type; (2) strains producing 'A' spores alone in standard medium, 'B' spores were formed with concentrations reaching 128 gm. per litre, except in the case of *Diaporthe*, 159; (3) with *P. citri*, J 1, which forms 'A', 'B', and 'C' spores in standard medium, 'B' and 'C' spores increase and 'A' spores correspondingly decrease numerically, 'B' spores predominating; (4) dimensions of spores increase by a significant amount.

In contrast with sugar, an increase in nitrogen content of standard medium does not in any circumstances induce formation of 'B' spores, and with strains producing spores of 'A' and 'B' types, 'B' spores decrease numerically. The tendency to check formation of 'B' spores may be overcome by increasing the sugar content of media rich in asparagin. Length of 'B' spores decreases significantly with increasing asparagin. 'A' spores become vacuolated with concentration reaching 3 gm. per litre and atrophied at higher concentrations.

Variations in proportions and dimensions of different kinds of spore associated with varied acidity or alkalinity and with laboratory conditions of temperature or illumination were not significant.

This work was carried out under the supervision of Dr. A. S. Horne, to whom the author is greatly indebted for valuable help and criticism. The author's thanks are also due to Professor V. H. Blackman for the facilities given and to Dr. F. G. Gregory for his advice on statistical methods. The author is indebted to Mr. H. Tooley for the photographic illustrations given in the paper.

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The Cytology and Life-history of *Sorosphaera* *Veronicae*.¹

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With four Figures in the Text.

INTRODUCTION.

THE type of nuclear division in the *Plasmodiophorales* has been the subject of discussion since Nawaschin (5) described the so-called cruciform stage in *Plasmodiophora Brassicae*. Although Nawaschin described this division as indirect, or mitotic, later workers have variously described it as amitotic and protomitotic. The general view on the life cycle has been that the somatic phase is diploid, but Osborne (6) and Horne (3) disagree with this and with the assumption that fusion takes place between motile elements.

Horne (3) alone gives adequate evidence in support of his views, and has counted the somatic chromosomes. It will be seen from the following account that the cytological details described by Horne, for *Spongospora*, are very similar to those of *Sorosphaera Veronicae*.

MATERIAL AND METHODS.

The material was collected by Dr. E. J. Schwartz in Kent. It was not fixed in the field on account of the extremely low temperature prevailing, but the galls were warmed for about an hour, cut up, and fixed in La Cour's 2 B fluid; various other fixatives were tried but were not so suitable. The material was passed into paraffin through chloroform, and sections were cut at 5-25 μ , 15 μ being found best. Staining was carried out by a modification of Newton's gentian violet-iodine method. A green filter was used throughout for the examination of the material and all drawings were made with a camera lucida.

¹ Thesis approved for the degree of Master of Science in the University of London.

OBSERVATIONS.

The somatic phase.

The nuclear divisions were found to be much more frequent than is usual in the *Plasmodiophorales*. Cook (2) calculates that in *Ligniera* the nuclei of one plasmodium in five thousand were dividing, while here the frequency was about one in fifty. The interphase nuclei are characterized by a conspicuous membrane immediately within which lie small chromatic granules connected with each other by fine threads. It is doubtful whether these threads would be visible in preparations stained with iron-haematoxylin owing to the fact that the cytoplasm is rendered dense and somewhat granular.

The nucleolus, termed the karyosome by many authors, is large, stains heavily, and occupies a more or less central position in the nucleus. Horne (3) shows the nucleolus as being connected to the membrane by fine threads, but these were not seen in *Sorosphaera*, probably because of the difference in staining methods.

The first indication of nuclear division is an increase in the staining properties of the chromatin (Fig. 1 *a*). This is probably due to an increase in chromaticity and to a contraction of the threads. The small granules on the membrane become obviously aligned (Fig. 1 *b*) and if the connecting threads had not been seen their presence would have been almost a certainty on this account. These early stages are not figured nor described by Horne (3) and would be placed between his figures 1 and 7. A further contraction of the chromatin threads occurs before they move away from the periphery. Distinct chromosomes could be resolved occasionally, and in one case (Fig. 1 *c*) there were clearly four. Still further contraction made the chromosomes fairly obvious, and several nuclei distinctly showed four in the nuclear cavity (Fig. 1 *d*). Even at this early stage there was a tendency for the the chromosomes to be arranged in one plane, very little movement taking place between this stage and metaphase. The profile view of this stage is figured by Maire and Tison (4, Figs. 18, 19) and described as the separation of the idiochromatin.

It will be noticed that until this stage the nucleolus remains spherical, but in later stages is elongated in the direction of the poles. The ends of the chromosomes can often be seen to be double at early metaphase, this being the first stage at which duality was observed. They are usually broadly U-shaped at metaphase, with the free ends pointing inwards (Fig. 1 *k*). There were, of course, many exceptions to this arrangement, but the majority of clear cases showed a strong resemblance to the nuclei figured by Horne (3, Figs. 21-8). At a rather later stage the typical configuration termed the 'Saturn stage' by Osborne (6) was seen. No indication of chromosome duality was apparent in the profile, but split

ends could be seen clearly in the polar view. Polar view was always difficult to resolve, the long thready chromosomes being very much tangled.

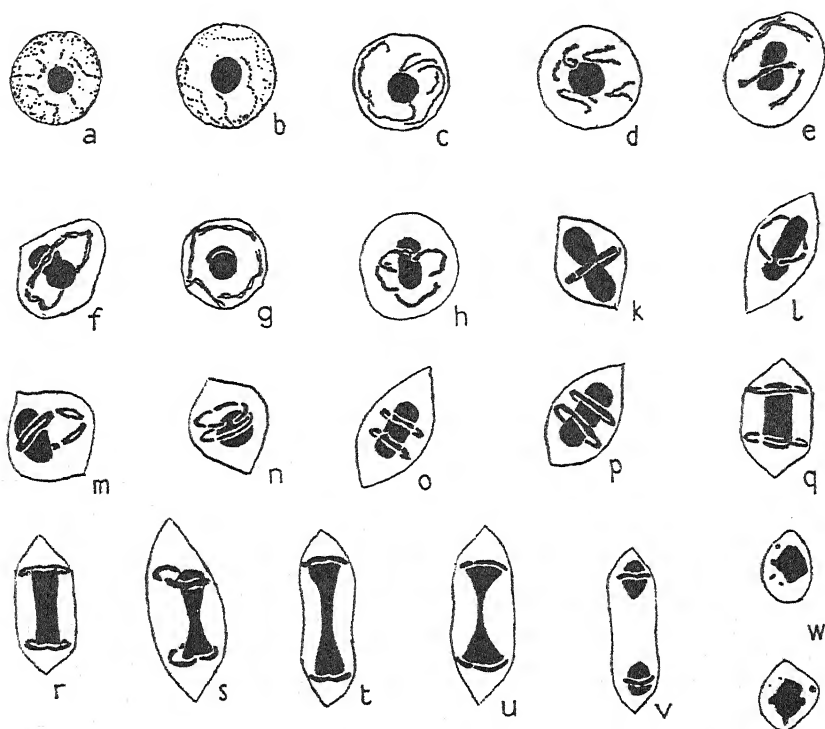


FIG. 1. The somatic mitosis ($\times 3000$).

The nucleolus was not always elongated in the polar direction, but was sometimes spherical and sometimes considerably out of its usual central position.

The chromosomes contract considerably during the long metaphase, and at early anaphase (Fig. 1 *m*) are much more widely separated than at early metaphase (Figs. 1 *k* and *l*). It may be inferred from Fig. 1 *m* that the spindle fibre attachment is median, for the median portions of the chromosomes are separated while the ends still cohere.

There are two pairs of chromosomes in *Sorosphaera*, one pair being distinctly longer than the other. They are not arranged in any definite order, for of twenty nuclei examined, twelve showed one of the possible arrangements and eight the other. Figures 1 *k* and 1 *m*, for instance, show the different arrangements, the two short chromosomes being adjacent in the latter.

A constriction appears in the nucleolus as a rule at telophase (Figs.

1 *q-u*) and finally a break occurs (Fig. 1 *v*) giving rise to a condition in which there are two groups of chromatin round the two halves of the nucleolus, one at each end of the nucleus. Lastly, the nuclear membrane constricts and divides in much the same way as the nucleolus, giving rise to two daughter nuclei in which the chromosomes adhere at first to the nucleolus (Fig. 1 *w*). Neither centrosomes nor asters were seen at any stage in the division. It will be noted that Horne does not figure centrosomes in the vegetative nuclei of *Spongospora*, with which fungus his work is chiefly concerned. Schwartz (7) has not seen them in the vegetative nuclei in *Sorosphaera* or *Ligniera*. The spindle fibres appear to follow the outline of the nuclear membrane, and are in consequence not at all obvious. This fact was recorded by Horne (3, p. 208) for *Spongospora*.

Fully developed plasmodia might contain as many as 32 nuclei. This, however, was the highest number recorded for this phase of the life-history, and was rather more infrequent than from 24 to 28.

The transitional phase.

This term is used by Horne (3) to cover the stages immediately prior to the so-called akaryote stage and the subsequent stages leading to the formation of a nucleus of more normal appearance. The changes observed in *Sorosphaera* are of a simple nature, consisting of the degeneration of the large and conspicuous nucleolus with the subsequent appearance of granules in the cytoplasm. At no stage was the normal 'reticulum' absent as some of the early workers, notably Osborne (6), suggest, but remained clearly visible against the nuclear membrane. It must be remembered that the cytoplasm in gentian violet preparations is very transparent, and it is quite possible that this fact alone accounts for the difference from the observations of workers who have used haematoxylin, which stain renders the cytoplasm rather opaque at this stage.

The nuclei were often associated in pairs, and were very variable in size. An attempt was made to count the numbers in the plasmodia, but it was found so difficult that the figures were of doubtful accuracy. It was believed, however, that the number might be between 14 and 30, and as far as could be seen there was no tendency to a maximum at any point between these figures. It can only be stated, then, as a result of this count, that the number is variable between wide limits. The appearance of paired nuclei at first suggests that a nuclear fusion takes place at this stage, but it was noticed that the paired nuclei were often larger than the unpaired, which is the reverse of the condition which might be expected if the solitary nuclei were diploid. Osborne's (6) statement that a fusion takes place at this stage is based upon this appearance of paired nuclei, and would have been more acceptable had it been supported with a chromosome count. The irregular masses of chromatin figured by most

workers probably belong to nuclei at a slightly later stage, for the number in the plasmodium is rather lower than in the stage described above, and is rather higher than the following stages, lying between 12 and 18.

The chromatin becomes dispersed over the membrane in a manner reminiscent of interphase, and two centrosomes and asters and a very large nucleolus appear. The nucleolus is even larger than that seen in the vegetative nuclei, but it is not by any means as dense.

Meiosis and spore formation.

At the end of the transitional phase the plasmodium is approximately spherical, and the chromatin in the nuclei accumulates in tangled masses at the poles. No signs of the splitting of a single centrosome were seen, there being two such bodies associated with each nucleus from the stage at which they are first visible. The appearance of centrosomes at this stage agrees with Horne's (3) account of *Spongospora*, but contrary to his results, however, it was not found possible to resolve eight chromosomes prior to pairing. In *Sorosphaera* pairing takes place between uncontracted threads.

After the appearance of two polar groups of chromatin in the nucleus (Fig. 2*d*), the nucleolus becomes flattened against the nuclear membrane and the two groups of chromatin move together (Fig. 2*e*). The configuration is very much like the collapsed zygotene (synizesis) commonly seen in fixed material in higher plants, and is probably the stage at which pairing takes place in this fungus. The following stages are, up to a point, strongly reminiscent of the corresponding stages in higher plants. The chromatin, which can now be seen to consist of beaded threads, spreads over the periphery and presents an appearance like the normal pachytene (Fig. 2*f*). The threads can occasionally be seen to be double, and when further contraction has taken place, four chromosomes can be resolved (Fig. 2*g*). This stage corresponds to diplotene in higher plants. Early diakinesis (Fig. 2*h*) and diakinesis (Fig. 2*k*) were both seen, and are of the type usually observed in higher plants. The fact that four well-defined bivalents were counted proves beyond all doubt that, the number of chromosomes in the vegetative nuclei being four, a nuclear fusion must have taken place during the transitional phase. The bivalents take up their position on the equatorial plate very much earlier than is the case in higher plants, longitudinal contraction continuing to a considerable extent after this has taken place in this instance, while in higher plants the highest degree of contraction is reached with the pairs scattered round the periphery. The nuclear membrane, however, persists for some time in *Sorosphaera* after the movement of the chromosomes to the plate.

The bivalents at heterotypic metaphase are not strained in the polar direction, but tend, like the chromosomes in the vegetative mitosis, to lie

closely in the equatorial plane. As far as the author is aware, this state of affairs is unique, and renders the profile of the division quite unrecognizable as a heterotypic metaphase. In polar view the structure can be resolved

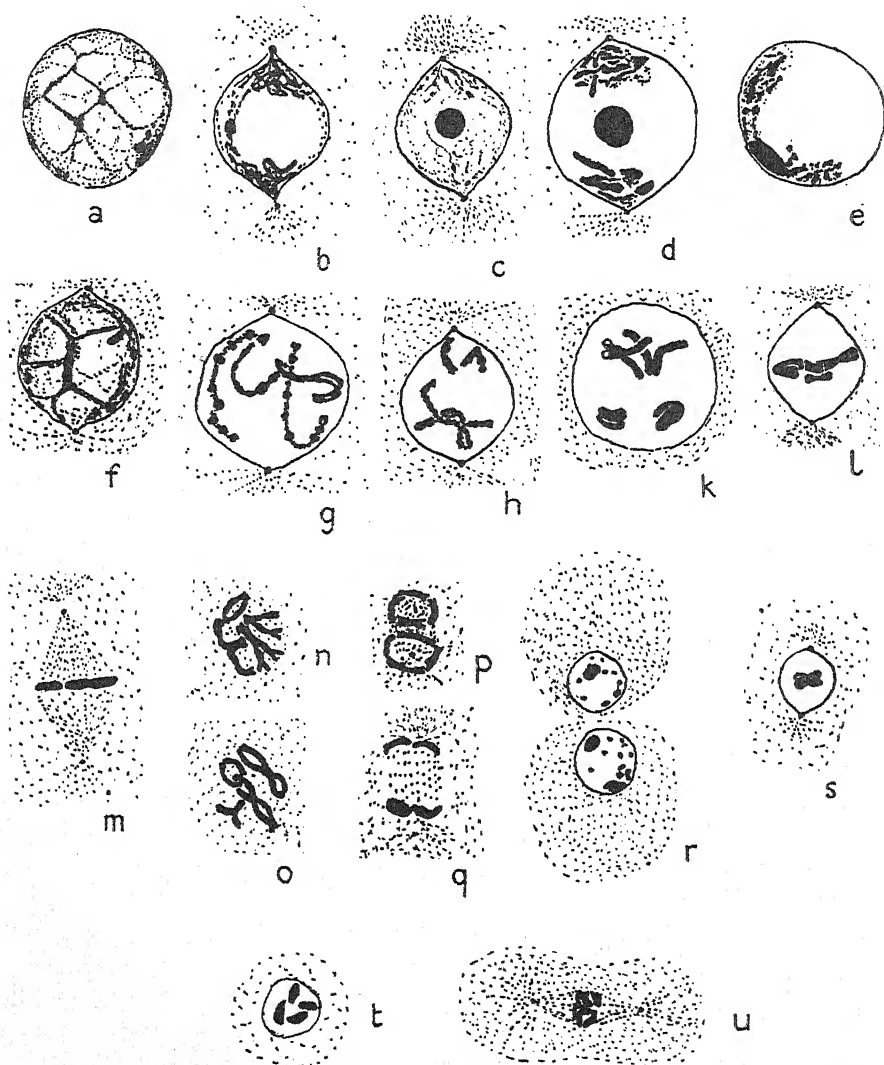


FIG. 2. *a-g*, heterotype division; *r-u*, homotype division ($\times 3000$).

only with difficulty, owing to the fact that the pairs usually lie, partly overlapping, in one plane. Where two chromosomes overlap, but are separated by a considerable depth of focus, they are easily resolved by carefully adjusting the focus. Where the two chromosomes are in the same focus

this is not possible, and an extensive search has to be made for a nucleus in which they are widely spread over the plate. It can often be seen that the free ends of individual chromosomes are split, the halves being widely

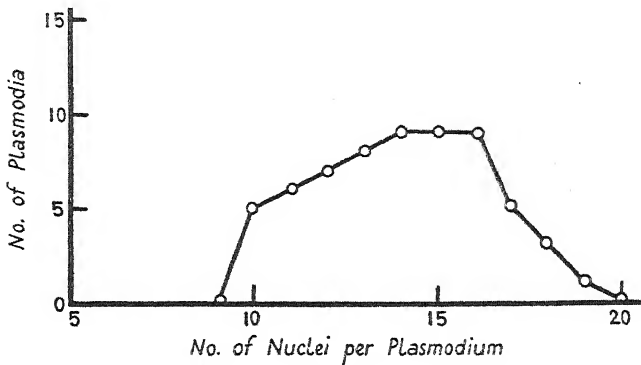


FIG. 3.

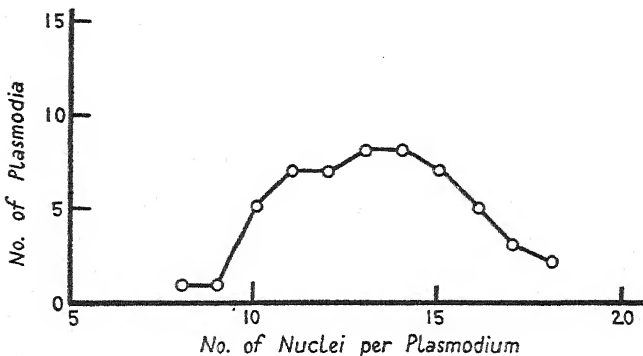


FIG. 4.

FIGS. 3 and 4. 3. Number of nuclei in plasmodia at diakinesis. 4. Number of nuclei in plasmodia at metaphase.

separated in a manner characteristic of a heterotypic metaphase (Fig. 2 *u*). In view of the coplanar arrangement of the chromosomes, however, it was felt that it would be necessary to give further proof that this metaphase followed the observed diakinesis. A search was therefore made for anaphases which could follow diakinesis without the interpolation of the metaphase, it being remembered that the chromosomes are already arranged on the plate at diakinesis. Such stages were not found. A count of the numbers of nuclei in the plasmodia at diakinesis was made (Fig. 3) and compared with a similar count at metaphase (Fig. 4). The two graphs are so similar that there is very little doubt that the two stages are part of the same division. Finally, stages between diakinesis and metaphase

were found, showing the breakdown of the nuclear membrane and the appearance of conspicuous spindle fibres (Fig. 2 *m*), which appear to move inward from the periphery until they present the profile shown in the above figure. It is, perhaps, worthy of note that the interlocking of bivalents is quite possible and may contribute to the difficulty of resolution of this stage. A suspected case of interlocking is shown in Fig. 20.

Early anaphases were not seen, probably owing to the extreme rapidity with which the chromosomes separate after the long metaphase. Telophases were frequent, though not nearly so common as metaphase. The general appearance is quite unlike that in *Spongospora*, judging from the figures given by Horne (3), for the nuclear membrane is definitely absent and the chromosomes are usually placed more or less end to end in *Sorosphaera* (Figs. 2 *p*, *q*, *r*). It is clear, however, in many nuclei, that the ends of the chromosomes are split (Fig. 2 *p*), this being characteristic of heterotypic telophases where the chromosomes have median attachment constrictions.

During the heterotypic division the plasmodium segments into uninucleate portions by the action of cleavage planes and surface furrows, as is usual in the lower fungi. These segments divide, by the same methods as does the plasmodium in the original cleavage, after the commencement of interkinesis, and the homotypic division takes place in these smaller uninucleate segments. Interkinesis was not at all common, and was therefore believed to be of short duration. This, of course, is to be expected, the chromosomes already having a visibly dual structure at heterotypic metaphase. The chromosomes do not appear to become very diffuse (Fig. 2 *s*), and there is thus no long prophase to the homotypic division. The chromosomes in this division are small and highly contracted (Figs. 2 *s-u*) before metaphase, and the nuclear membrane persists until after the metaphase plate has been formed. The general appearance in the early stages is not unlike that at the corresponding stage in the heterotypic division, the thread stages being absent, and the metaphase is also distinguishable chiefly by the form of the chromosomes and the number of nuclei in the plasmodium. The nucleus is, of course, much smaller than the diploid, but the size of fungal nuclei is so variable that no use was made of this fact in placing the stage. Early anaphase (Fig. 2 *u*) shows four chromosomes which have separated first in the median portion, no doubt owing to the fact that the spindle fibre attachment is median.

A further cleavage of these binucleate segments takes place, and the uninucleate portions so formed round off into a hollow sphere. A thick wall is laid down round each spore, and the spore ball becomes regular in shape. Finally, the whole ball is covered with a thin envelope, and on an average contains about 70 spores. This number was arrived at by a calculation based on the distance between the nuclei in a median section of a sphere

of average size. This figure was checked by a direct count, and agrees roughly with the figure to be expected from the homotypic division, which would give an upward limit of 64. Small groups of spores were occasionally seen, and there were also a few examples of double spore balls. The spore balls were usually to be found near the outside of the galls, and the younger stages rather nearer the centre, there usually being a small proportion of vegetative nuclei with the transitional and post-transitional nuclei. There were often several distinct areas of infection of different ages in the same gall, one case being found in which there were two, one of which contained nothing but vegetative nuclei and the other a large proportion of spore balls.

DISCUSSION.

On the evidence presented by Horne (3) and in this present work it must be admitted that true chromosomes appear in the vegetative divisions of two genera in the *Plasmodiophorales*, and it will be fairly safe to state that they will be found in the other genera if sufficiently active material can be obtained. Any speculations with regard to the affinities of this group which are based on the non-appearance of chromosomes must, therefore, be viewed with suspicion.

There are several peculiar features in the division, notably the persistence of the nucleolus, and the fact that the whole process is intranuclear. It has also been noted in the present work that the metaphase chromosomes lie unusually close to the equatorial plane, but so little is known about the cytology of the fungi, with the exception of the Ascomycetes, that it is very unsafe to make use of nuclear phenomena in their classification. The persistent nucleolus is not peculiar to the *Plasmodiophorales*, for, according to Belar (1), the same persistence occurs in *Chlamydomorphys schaudinni*. Darlington ('Recent advances in Cytology'), commenting on Belar's account, writes: 'We need not ascribe any special virtue to this persistence; conditions external to the nucleus such as might determine a rapidity of prophase changes or a greater viscosity of the nucleus, as Belar suggests, may be held responsible.' The term 'karyosome' has been avoided in this work on account of the variety of shades of meaning ascribed to it in the work of various authors. Apart from the fact that it does not disappear before metaphase, but is passed on to the daughter nuclei, there is no evidence that this body is anything but the nucleolus present in most nuclei, for no regular connexion was seen between it and the chromatic threads, neither did the threads occasionally seen in contact with it stain any more deeply than those which were not.

It seems strange that Maire and Tison (4) should not have observed that there were four distinct groups of chromatin at prophase, for they figure very clear profiles of this stage. Cook (2) does not recognize any

stage earlier than that at which, according to him, the chromatin is aggregated to form a ring round the periphery of the nucleus. Horne (3) both recognizes and resolves the prophases in *Spongospora*, counting four chromosomes, and his figures of this stage, and in fact of all the other stages in the mitosis, might equally well have been drawn from *Sorosphaera*.

The apparent ring at metaphase is very difficult to explain satisfactorily. The chromosomes are very clearly separate at prophase in this material, and those who would have us believe that they are regularly united end to end at metaphase would have great difficulty in the formulation of a theory to fit all the known facts. Horne (3) points out that in *Spongospora* the combined length of the chromosomes is approximately equal to the circumference of the nucleus. In *Sorosphaera* the combined length is considerably greater at early metaphase, but is slightly less at early anaphase, with the result that small gaps are visible between the chromosomes at the latter stage, but it is only in polar view that early metaphase can be resolved. The spindle fibres are attached to the median portion of the chromosome, and extend round the periphery of the nucleus. It is at once obvious that as soon as the fibres exert any force on the chromosomes, the latter will start to move toward the periphery of the nucleus, being held, at early anaphase, between the nuclear membrane and the nucleolus. The marked contraction of the chromosomes on the plate is unusual, and it would seem that the chromosomes move to this position very much earlier than is the case in most nuclei. No explanation of the apparently closed ring is possible on any grounds other than the purely mechanical causes referred to above. The possibility of chromosome association has been examined, and has been rejected for two reasons. Secondary pairing is, of course, entirely out of the question, and the type of association to be expected from the partial prevalence of meiotic conditions is equally impossible, for in the first place the chromosomes are arranged at random, and secondly, there is no tendency to ring formation at diakinesis. In the opinion of the author the failure of Cook and other workers to observe chromosomes is probably due to the examination of too few nuclei. Metaphase, by far the most common stage, does not show the disposition of the chromatin at all clearly in profile or in polar view, and the comparative rarity of the other stages renders inactive material useless. Gwynne-Vaughan and Williamson emphasize the importance of active material in the *Ascomycetes*, and their argument applies with equal force to the fungus under consideration and to the other members of the group.

The accounts of the so-called 'akaryote' stage are even more varied than those of the vegetative phase. Osborne (6) states that the vegetative nuclei disappear entirely, and that the new nuclei arise in different positions. The new nuclei are haploid, and a fusion takes place between pairs. According to Horne (3) the 'new nuclei' are already diploid, a fusion

having taken place, presumably during the period when there is no visible chromatin in the nuclei. Cook and Schwartz believe that the nucleus is never entirely devoid of chromatin, and that there is not a fusion during this phase of the life-history, the vegetative nuclei being diploid. It must be pointed out that a long diploid phase is extremely improbable in so primitive an organism, and that a nuclear fusion might be expected to take place in the akaryote stage on these grounds. With Cook and Schwartz, however, it is difficult to believe that the whole of the chromatin is extruded at this stage, for it is impossible that any of the essential hereditary material should be lost. The account given by the author shows that this difficulty does not exist in reality since it is now possible to trace the life cycle of one of the *Plasmodiophorales* through this stage without running contrary to the views of modern cytology. It only remains, then, in the consideration of this stage, to account for the appearance of stainable granules in the cytoplasm. These granules must result from the breakdown of the nucleolus, on the disappearance of which body all investigators are unanimous.

The later stages, in which bipolar groups of chromatin and configurations not unlike the zygotene of meiosis in higher plants are seen, suggest that the nucleus is diploid, but eight individual chromosomes could not be resolved. The behaviour of the chromatin threads in the stages described as pachytene, diplotene, and diakinesis are so typical of meiosis, however, that when four bivalents have been counted we are perhaps justified in assuming that the diploid number must be present. The only abnormality which could be found lay in the fact that the chiasmata were apparently not so strongly terminalized as they are in most plants. It was not felt that any statistics could be given on account of the difficulties of observation. Terminalization was complete at metaphase, which stage was not at first recognized as belonging to the heterotypic division on account of the coplanar arrangement of the chromosomes.

Previously to the publication of the work of Horne, no author had given convincing proof that meiosis takes place in the two divisions immediately prior to spore formation. Cook (2) states as evidence of a reduction in the chromosomes the fact that the plate of the second division is about half the size of that of the first. The variability in size of fungal nuclei is well known, and such evidence is therefore unreliable. Cook also states in the same paper that there is no true prophase to the second division, this being a characteristic of the homotypic metaphase in all plants except cases in which a restitution nucleus is formed. As the prophases to the vegetative division and to the heterotypic division are not described in Cook's paper, his statement that there is no prophase to the only remaining division can be of little value.

Turning finally to the life-history, we have a complete nuclear cycle,

with a fusion followed immediately by a reduction, the haploid number being four. This number agrees with that found by Horne (3) in *Spongospora*, the vegetative phase being haploid in both cases.

SUMMARY AND CONCLUSIONS.

The vegetative phase of *Sorosphaera Veronicae* is haploid, the chromosome number being four. The apparently enclosed ring at metaphase is caused by the retention of the chromosomes between the nuclear membrane and the nucleolus, both of which are persistent. The fact that the spindle is peripheral may also contribute to the unusual configuration.

The nucleolus disintegrates at the transitional stage, but the continuity of the chromatin is not lost. It is concluded from chromosome counts and other evidence that a nuclear fusion takes place during this stage.

Prophase stages in the reduction division showed four bivalents and were normal, but at heterotypic metaphase all the chromosomes become coplanar. This latter tendency was also noted in the vegetative mitoses.

The homotypic division follows quickly after the heterotypic, the normal number of nuclei in the segmented plasmodium immediately prior to spore formation being about 60.

Since this life cycle is not compatible with existing views on the infection, it is highly desirable that the latter should be re-investigated.

The thanks of the author are due to Professor R. R. Gates for his valuable help and criticism, to Mr. D. G. Catcheside for the confirmation of many critical stages, and to Dr. R. J. Schwartz for his kindly interest and for the excellent material.

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The Carbon Dioxide Carbohydrate Ratio in the Aerobic and the Anaerobic Respiration of Rice.

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With four Figures in the Text.

AS a result of studies in respiration of a population of ripening apples by Blackman and Parija (2) and Parija (11) under aerobic and anaerobic conditions, Blackman (1) has introduced new concepts into the process of respiration. According to him the reserve carbohydrates by hydrolysis give rise to normal hexoses which are converted into heterohexoses, and these heterohexoses supply the substrate for glycolysis, which in turn supplies the substrate for respiration. According to Blackman glycolysis should be regarded as the common measure of respiration in all conditions, and the heterohexoses and not the normal hexoses should be considered the substrate for oxidation.

Gustafson (9) has studied the anaerobic respiration of tomato fruits, and attributed the increased production of carbon dioxide in air after the anaerobic treatment of the tomato fruits to the production of some substance in intramolecular respiration.

Ermakoff and Iwanhoff (8) studied the respiration of oil-bearing seeds, and they found that monosaccharides and disaccharides increased at the beginning of germination, but the oil content remained unchanged during the first day of germination and decreased on the second and third day, thus indicating that the germinating seeds in the oil plants used carbohydrates as respiratory material.

Ranjan (12) has shown that in tropical plants the sugars of leaves kept in nitrogen are less than those of leaves in air.

In order to enlarge our knowledge of this intricate problem of plant respiration it is necessary to measure the output of carbon dioxide of a

plant organ under anaerobic and aerobic respiration at different stages of its life, and to make a carbohydrate analysis before and after the exposure to aerobic and anaerobic conditions. As Blackman (2) states, such a study of carbohydrate change is being made for apples at Cambridge, but so far no results have been published. The results obtained with apples can hardly be comparable with the results obtained with germinating seedlings, as in the former case there is no consumption of materials in growth, while in the latter case side by side with the utilization of carbohydrates for respiratory purposes, a certain amount is used for the growth of the seedlings. What is wanted is a comparison of the rates of respiration, aerobic and anaerobic, with the amounts of different carbohydrates consumed, and an observation of the differences in the growth made by the seedlings under the two conditions of germination. It is likely that the respiratory phenomena in a population of ripening apples will be different from that in the growing plant.

Rice was selected as the material for such a study, since the seed can be readily germinated throughout the year, the reserve material is mostly starch, and there is a negligible amount of protein and fatty materials.

Measurement of Aerobic Respiration.

In this investigation the respiration of rice seedlings is measured by a special apparatus. The apparatus used for measuring both aerobic and anaerobic respiration was the same, excepting that in the former case the air, deprived of its carbon dioxide, was aspirated through the glass chamber, in which the respiratory material was kept, by means of a water pump attached to the absorption apparatus, while in the latter case nitrogen gas from a cylinder was passed through the apparatus. In Fig. 4 the apparatus as used for the determination of anaerobic respiration is shown. The seedling chamber consisted of a cylindrical glass jar (R), 10 inches in height and 5 inches in diameter. The seedlings were kept on a moistened filter paper, below which was placed a thick pad of cotton sufficiently soaked in water. This arrangement avoided the difficulty of watering the seedlings, especially where measurements of respiration were made with the same material throughout the ten days. The temperature inside the chamber was kept constant at 30° C. by a regulator (G).

The carbon dioxide in the air leaving the chamber, at the rate of 100 litres an hour, was determined by absorption in a Reiset tower containing 0.4 N NaOH solution.

In the beginning respiration in air was studied with only 25 seedlings; but later it was found that with 200 seedlings the experimental error was negligible. The rate of respiration was measured during six hours, taking hourly readings in all the experiments, and the mean of the six readings

taken as the output of carbon dioxide (mg. per hour). After each experiment the rice seedlings were dried in an oven at 100°C . for a period of forty hours. In all, three sets of experiments were carried out. In one set

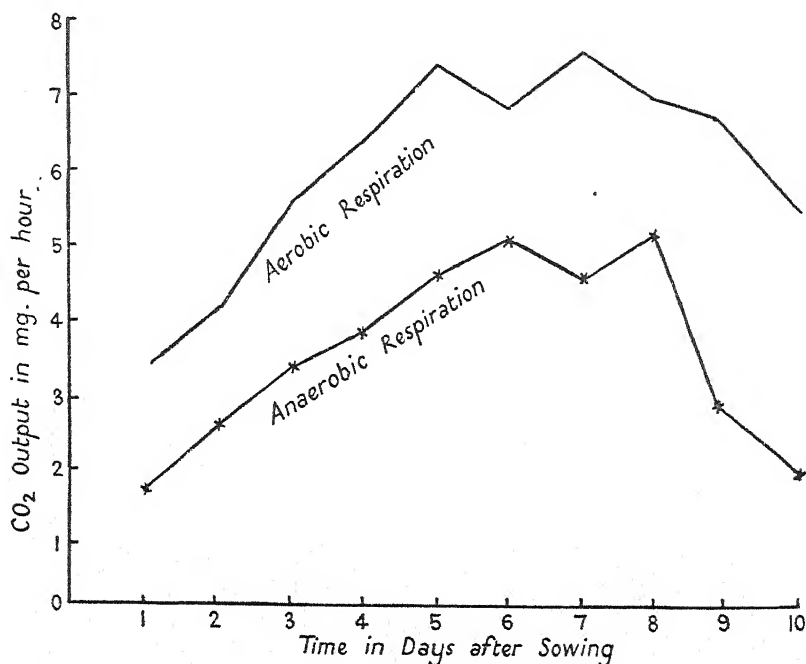


FIG. 1. Graphs showing the rates of aerobic and anaerobic respiration of the seeds of rice germinated in the dark. (A new set of 200 seedlings was used each day.)

of experiments rice seedlings germinated in the dark (dark series), and 200 were removed every day for the measurement of respiration, the whole experiment being continued for ten days. In the second set of experiments seedlings were germinated in the light (light series). In these two experiments the daily respiration readings were made with fresh material each day. In the third set of experiments, however, the rate of respiration of the seedlings in air was measured for ten days with no change of material. In this set it was, of course, not possible to determine the dry weights every day, and to get over this difficulty a separate set of seeds was germinated in the dark simultaneously, and the dry weights of 200 seedlings were taken every day after the rate of respiration of the seedlings in the chamber had been measured.

Fig. 1 shows the rate of respiration of the seedlings during ten days' germination in air in the dark with daily change of material. The rate of respiration rises at first, reaching its maximum on the fifth, sixth, or seventh day, and then it begins to fall.

Fig. 2 gives the rate of respiration of the seedlings in air in the dark for ten days with no change of material. But then the maximum rate of respiration is reached about the same time as in the previous set.

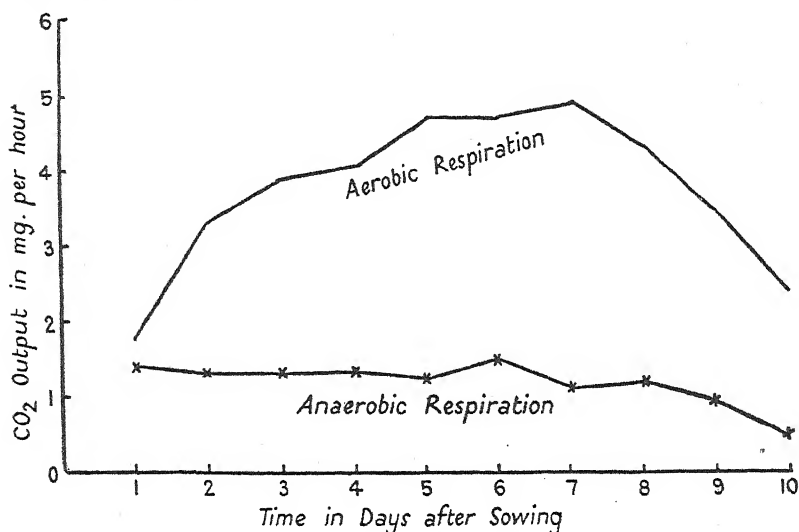


FIG. 2. Graphs showing the rates of aerobic and anaerobic respiration in seeds germinated in the dark. (The same set of 200 seedlings was used throughout.)

Fig. 3 gives the results of the carbon-dioxide output of the seedlings germinated in the light. The carbon-dioxide output increases and reaches its maximum on the sixth and the seventh day, and then diminishes. There are marked differences in the respiratory rates in the two cases.

Anaerobic Respiration.

In this investigation compressed nitrogen in a steel cylinder fitted with two reducing valves with two pressure gauges was used. The nitrogen gas is first passed through two soda-lime U-tubes (A) and two towers containing potassium hydroxide solution (K) and alkaline pyrogallous acid (P) respectively (Fig. 4), as it contains about 0.1 per cent. of carbon dioxide and also oxygen. In all the experiments the nitrogen gas was bubbled through the respiratory chamber very rapidly for an hour to drive off air from the chamber and to replace it by nitrogen. After an hour the rate of nitrogen gas coming out from the cylinder was slowed down and was kept at from 1 to 2.5 atmospheres, or nearly 40 litres, per hour.

The anaerobic respiration of rice seedlings was similarly measured in three ways. In one set of experiments the seedlings germinated in the dark in air were kept in the respiration chamber, and hourly readings of carbon-dioxide output were taken in the same way as in the aerobic respiration series. This will give a measure of the carbon-dioxide pro-

duction under anaerobic conditions during the same period when aerobic output of carbon dioxide was measured. This will give an idea of the difference in the carbon-dioxide production under aerobic and anaerobic

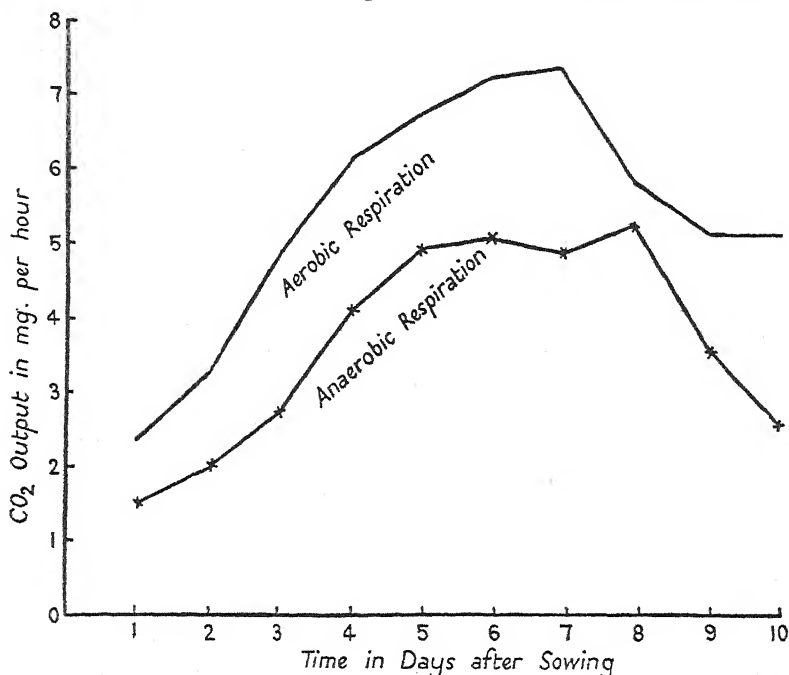


FIG. 3. Graphs showing the rates of aerobic and anaerobic respiration in seeds of rice germinated in light. (A new set of 200 seedlings was used each day.)

conditions by the seedlings grown in air in the dark and with daily change of material.

In the second set of experiments the seedlings were germinated from the very beginning in the atmosphere of nitrogen, and after every twenty-four hours the output of carbon dioxide was measured as before. The nitrogen gas was allowed to bubble day and night for ten days without stoppage. After the tenth day the rice seedlings were taken out of the respiratory chamber and photographed immediately to show the differences between the seedlings grown in air and nitrogen continuously. It was observed that in nitrogen the radicles did not come out of the seeds till the tenth day.

In Fig. 1 the results of the carbon-dioxide production in nitrogen of the seedlings grown in air in the dark with daily change of material are given. It will be seen by comparing the graphs that there is a slightly depressed respiratory rate in nitrogen, but the rise and fall in the carbon-dioxide production during ten days resemble those obtained for the carbon-dioxide output of the seedlings in air.

For the seedlings grown in nitrogen from the very beginning, the

carbon-dioxide production was very much below the values obtained either with seedlings grown in air with or without change of material or with seedlings grown in air and the respiration measured in nitrogen (Fig. 2).

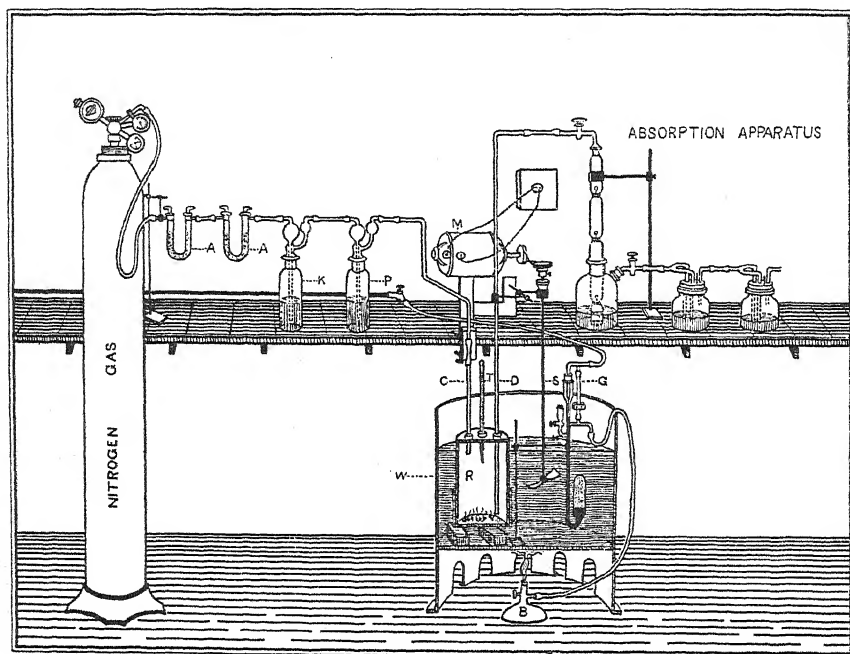


FIG. 4. Diagram of the apparatus used for measuring anaerobic respiration in longitudinal section.

There is no appreciable rise or fall in the carbon-dioxide production during the first eight days, after that the carbon-dioxide production appears to decline.

As in the case of aerobic respiration, the seedlings were also grown in air in the light, and the rate of anaerobic respiration was measured with daily change of material. On comparing the graphs given for aerobic respiration of the seedlings given in Fig 3, it will be noticed that the rate of respiration is lower in nitrogen than in air. With daily change of material the maximum rate of respiration in nitrogen is reached in the dark as well as in the light series on the eighth day of germination.

The measurements of aerobic and anaerobic respiration are made in three different ways, the aerobic and anaerobic rates of respiration of the seedlings germinated in the dark and in the light with daily change of material, and with no change of material. It is therefore possible to determine the magnitude of the ratio of the intensity of anaerobic respiration to that of the normal respiration. The values of the ratio (I/N) vary with different

plants. Except in the case of seedlings of *Vicia Faba* the I/N was always less than unity, and fluctuated between 0.24 and 0.49, in the case of seedlings of different species. Recently Boysen-Jensen (see 10) has carried out determinations of I/N of roots, of grapes, of leaves of *Tropaeolum majus*, of potato tubers, and the seedlings of *Sinapis* sp. In the case of grapes the I/N was more than unity, while in seedlings and other plant parts I/N was less than unity.

The determinations of I/N for the rice seedlings at different stages of germination were calculated from the results obtained above. The point of difference from similar determinations made by previous workers is that in this investigation there are continuous records of the rates of respirations in air and in nitrogen, and so it is possible to study the changes in the I/N ratio at different stages. Table I gives the I/N ratio for the rice seedlings germinated in dark and in light, with daily change of material, and during ten days.

TABLE I.

Respiration of Rice Seedlings Germinated in the Dark and in the Light.

The Results are given in mg. CO₂ in Six Hours per 200 Seedlings.

N = Aerobic and I = Anaerobic Respiration.

	Germinated in dark.			Germinated in light.		
	N.	I.	I/N.	N.	I.	I/N.
1st day	19.5	9.2	0.48	14.4	9.1	0.63
2nd "	23.7	14.5	0.60	19.8	11.9	0.60
3rd "	32.4	18.9	0.58	29.3	15.1	0.51
4th "	38.6	22.4	0.55	36.5	24.4	0.67
5th "	43.5	26.0	0.59	40.1	29.1	0.72
6th "	41.3	29.2	0.70	43.0	30.1	0.70
7th "	44.5	26.4	0.59	43.6	28.9	0.66
8th "	40.9	30.1	0.73	34.9	31.1	0.89
9th "	38.8	16.0	0.41	30.9	21.2	0.69
10th "	31.5	11.2	0.35	30.1	15.3	0.50

Here also the value of I/N at any stage of germination is less than one. In the dark series the I/N value is low in the beginning and increases up to 0.73 on the eighth day, after which it falls. The same sequence holds good in the case of the light series, but the values of I/N differ here and there from the corresponding values of I/N in the dark series.

In Table II the values of I/N for the rice seedlings grown in air and in nitrogen without change of material are given.

Here the values for I/N are low. As the rice seedlings are germinated in an atmosphere of nitrogen for ten days, the output of carbon dioxide is very low. Often for the first two days the values of I/N are fairly uniform.

TABLE II.

Respiration of Rice Seedlings Germinated in Air and in Nitrogen. In each Case the same Set of 200 Seedlings was used. Data in the same Terms as Table I.

	N.	I.	I./N.
1st day	10.4	8.4	0.81
2nd "	19.4	7.9	0.40
3rd "	23.5	6.2	0.26
4th "	24.6	7.2	0.29
5th "	28.3	5.8	0.20
6th "	28.0	8.9	0.31
7th "	29.1	9.5	0.32
8th "	25.6	7.5	0.29
9th "	20.9	5.6	0.27
10th "	14.3	2.7	0.27

The average values of I/N ratios in the case of anaerobic respiration with daily change of material (Table I) germinated in the dark and in the light are 0.57 and 0.67 respectively, and that of the I/N ratio with no change of material (Table II) is 0.31.

Extraction of Carbohydrates.

Rice seeds (Columba, variety 42) were first soaked in water for a period of sixteen hours and then sown. It was noticed from preliminary experiments that well-soaking the seeds in water facilitates satisfactory germination. The soaking period, as mentioned above, was the same for all the experiments in this investigation. For the dark series the seeds were sown for germination on moist blotting-papers in a series of germinating dishes, which were kept in a closed dark room but exposed to air. For the light series the seeds were sown in a moist sawdust in diffuse sunlight.

Every day 200 seedlings were removed for the determination of carbohydrate. They were quickly weighed for fresh weight, and were used for the extraction of sugars, according to the method used by Davis, Daishi, and Sawyer (5).

Determination of Sugars and Starch.

The method of estimating sugars as first used by Folin and Wu, and later improved upon by Calvert (3), Stanford and Wheatley (13), and recently by Dastur and Samant (6), was employed. The two solutions—copper sulphate solution and phosphomolybdic acid solution—were prepared according to Calvert (3), and the hexoses, cane-sugar, and starch were determined according to the method of Dastur and Samant (6).

The analysis of carbohydrates in the seedlings was made every twenty-four hours for the ten days of germination. The hexoses, cane-sugar, and starch are determined and calculated as hexoses. Though the rice seeds contain about 70 per cent. of their dry weight as starch, it is a curious fact that in no case has maltose been found during the investigation. In Table III the sugars and starch are calculated as percentages of dry weight. The weight of the sugars is added to the dry weight of the material after extraction with alcohol to give the dry weights here recorded; they do not include any protein matter and fatty substances removed along with the sugars by extraction with alcohol.

TABLE III.

Weights (gm.) and Carbohydrates of Rice Seedlings Germinated in the Dark in Air. The Carbohydrates are given in Terms of 100 gm. of Dry Weight; the Starch and Cane-sugar are in Terms of Hexoses.

Days of germination.	Fresh wt.	Dry wt.	Hexoses.	Cane-sugar.	Starch.
1st day	3.70	2.494	0.015	0.535	73.25
2nd "	4.8	2.362	0.090	2.050	70.62
3rd "	6.9	2.097	0.312	2.563	67.26
4th "	8.08	1.924	0.382	5.471	59.30
5th "	9.14	1.976	4.103	5.355	47.23
6th "	10.99	1.890	2.019	5.960	40.87
7th "	11.79	1.802	1.412	6.219	37.3
8th "	11.29	1.681	1.533	6.645	35.3
9th "	13.53	1.498	0.146	3.684	33.41
10th "	14.35	1.456	0.190	4.935	27.10

The results of the carbohydrate analysis show that sugar appears as the seedling germinates and the starch content decreases. The maximum amount of sugar is found from the fifth day of germination to the eighth day of germination, and then the quantity decreases.

Table IV gives the results of carbohydrate analysis of the rice seedlings germinated in the light in air. The results are calculated as percentages of dry weight.

The quantity of sugars produced increased as germination proceeded, as in the case of the seedlings grown in the dark, but the actual amounts of sugars present at each stage of germination are less. On the other hand, the starch contents fall more rapidly in the light series than in the dark series. This is unexpected, as the output of carbon dioxide is greater in the seedlings grown in the dark than in the seedlings grown in light. It is possible that in the light series there is a greater consumption of carbohydrates for the purposes of growth than in the dark series.

An analysis was made of the seedlings grown in air but whose rate of respiration was measured in nitrogen. In this case the analysis of carbohydrates was made eight hours later than in the previous two cases of the

seedlings mentioned above. This gives a measure of the carbohydrates consumed under anaerobic conditions during eight hours, as the carbohydrate analysis is made before and after the measurement of anaerobic respiration.

TABLE IV.

Weights (gm.) and Carbohydrates of Seedlings Germinated in the Light in Air. The Carbohydrates are expressed in Terms of 100 gm. of Dry Weight; the Starch and Cane-sugar in Terms of Hexoses.

Days of germination.	Fresh wt.	Dry wt.	Hexoses.	Cane-sugar.	Starch.
1st day	3.46	2.57	0.017	0.681	71.87
2nd "	3.84	2.45	0.026	0.958	69.72
3rd "	4.63	2.35	0.079	2.247	66.45
4th "	5.96	2.23	0.174	3.846	57.80
5th "	6.97	2.036	0.259	3.599	49.67
6th "	7.91	1.93	0.148	3.671	43.03
7th "	8.59	1.85	0.480	3.673	37.43
8th "	9.35	1.79	0.365	3.298	32.88
9th "	10.51	1.66	0.045	4.963	21.78
10th "	12.74	1.65	0.024	2.727	20.86

A similar analysis was made of seedlings grown in air in the light, and treated anaerobically for a period of eight hours, i.e. from twenty-four hours to thirty-two hours, forty-eight hours to fifty-six hours, and so on. The actual data, however, are not given.

With a knowledge of the concentrations of the different carbohydrates present at the end of the period of anaerobic respiration, and of the carbohydrates present before anaerobic treatment (given in Tables III and IV), it is possible to determine the total loss of carbohydrate in anaerobic respiration of the seedlings grown in air in light as well as in dark.

An analysis of the seedlings germinated in an atmosphere of nitrogen from the very beginning was made at successive stages of germination. The results are shown in Table V.

The most significant result of the above analysis was the total absence of hexoses at all stages of the seedlings; also the amount of cane-sugar produced did not increase appreciably.

From the results of carbohydrate analysis of the rice seedlings given above, it is possible to determine the carbohydrates consumed each day, and to determine the relations of the total loss of carbohydrates with the output of carbon dioxide during the same period. The loss of metabolic carbohydrate material cannot be due to respiration alone, as some of the carbohydrate material must have been used for the purposes of growth also.

The total metabolic carbohydrate loss can be determined by finding the difference between the metabolic carbohydrate contents of the seedlings on two successive days. The following Table VI gives the total loss of carbo-

hydrates as hexoses (mg.) and the carbon-dioxide production (mg.) in the seedlings grown in air in the light and in the dark. In order to show clearly the relations between the output of carbon dioxide and the total loss of carbohydrates as hexoses, the carbon dioxide carbohydrate ratios for aerobic and anaerobic respiration are calculated. If hexose is the sugar which is used as respiratory material, the carbon dioxide hexose ratio in aerobic respiration should be 1.48. In anaerobic respiration the ratio should be 0.48.

TABLE V.

Analysis of Rice Seedlings Germinated for Ten Days in an Atmosphere of Nitrogen. Cane-sugar and Starch are Expressed as Hexoses, and Concentrations are given in Terms of 100 gm. of Dry Weight.

Stage of germination.	Dry wt.	Hexoses.	Cane-sugar.	Starch.
1st day	2.411	Nil	0.5666	73.02
2nd "	2.448	"	0.5064	70.26
3rd "	2.336	"	0.6960	67.83
4th "	2.248	"	0.7182	67.30
5th "	2.290	"	0.8964	63.03
6th "	2.167	"	0.7327	63.00
7th "	2.116	"	0.9104	59.86
8th "	1.977	"	1.0124	59.24
9th "	1.957	"	0.9577	57.10
10th "	2.102	"	0.9303	52.26

Table VI gives the carbon dioxide carbohydrate ratio in the aerobic respiration of the seedlings grown in the dark and in the light, as calculated on a basis of 100 gm. of the dry weight of the seedlings.

TABLE VI.

The Carbon Dioxide Carbohydrate Ratio of Aerobic Respiration of Rice Seedlings grown in Light and in Dark.

Stage of germination.	Light series.			Dark series.		
	CO ₂ (mg. in 24 hrs.).	Loss of carbohydrates as hexoses (mg. in 24 hrs.).	Ratio.	CO ₂ (mg. in 24 hrs.).	Loss of carbohydrates as hexoses (mg. in 24 hrs.).	Ratio.
1st-2nd day	57.6	20.8	2.8	78.0	11.7	7.2
2nd-3rd "	79.2	17.0	4.6	94.0	25.2	3.7
3rd-4th "	117.2	69.5	1.7	129.6	49.8	2.6
4th-5th "	145.0	84.9	1.7	154.0	84.6	1.8
5th-6th "	160.0	66.1	2.4	173.6	78.3	2.2
6th-7th "	172.0	52.7	3.2	165.2	39.1	4.2
7th-8th "	174.4	50.9	2.7	178.0	14.5	12.0
8th-9th "	139.6	97.5	1.6	163.0	62.3	2.6
9th-10th "	123.6	30.6	4.0	155.2	50.0	3.1
Total	1168.6	490.0	Mean 2.38	1290.6	415.5	Mean 3.1

The relation between the carbon-dioxide production and the carbohydrate consumption during anaerobic respiration can be determined from the results given above, as was done in the case of aerobic respiration in Table V. This would show the differences in the carbon-dioxide production and the loss of carbohydrates in the aerobic and anaerobic respiration. The following Table VII gives the carbon-dioxide production and the loss of carbohydrates as hexoses during the period of anaerobic respiration of the seedlings grown in air in the light and in the dark.

TABLE VII.

The Carbon Dioxide Carbohydrate Ratio of Anaerobic Respiration of Rice Seedlings.

Stage of germination.	Dark series.			Light series.		
	CO ₂ (mg. in 6 hrs.).	Loss of carbohydrates as hexoses (mg. in 6 hrs.).	Ratio.	CO ₂ (mg. in 6 hrs.).	Loss of carbohydrates as hexoses (mg. in 6 hrs.).	Ratio.
24-32 hrs.	10.6	13.8	0.76	10.6	20.0	0.53
48-56 "	16.8	10.3	1.60	13.7	11.2	1.20
72-80 "	21.0	17.1	1.23	17.6	33.2	0.53
96-104 "	26.1	26.0	1.00	28.4	36.2	0.77
120-8 "	30.0	38.3	0.79	33.9	42.8	0.80
144-52 "	34.0	52.3	0.64	35.0	24.7	1.40
168-76 "	30.8	69.3	0.56	33.7	14.4	2.30
192-200 "	35.1	98.5	0.35	36.2	18.5	1.98
216-24 "	18.6	86.5	0.21	24.6	21.0	1.10
Total	223.0	412.1	Mean 0.54	333.7	222.0	Mean 1.6

The carbon-dioxide production and the carbohydrate contents of the seeds germinated in air and in nitrogen may be compared by taking the 200 seeds as the unit, since the dry weights of seedlings at each stage cannot be determined in the continuous series. Since we are concerned with the relative values of carbon dioxide and the carbohydrate contents, this method of comparison is satisfactory. The following Table VIII gives the carbon-dioxide production in mg., the carbohydrate contents as hexoses in mg., and carbon dioxide carbohydrate ratios of the 200 seeds germinated in air and in nitrogen with no change of material.

The total loss of carbohydrates each day is much lower in anaerobic than in aerobic conditions. The loss of carbohydrates is greatest on the second day in both cases. It will be seen from the Tables VI, VII, and VIII that the carbon dioxide carbohydrate ratio is greater than the theoretical ratio both in aerobic and in anaerobic respiration. If the results in Table VIII for the continuous series were calculated as percentages of dry weights of the seedlings at each stage of germination, the carbon dioxide carbohydrate ratios for aerobic and anaerobic respiration will be still higher than those calculated on the basis of 200 seedlings.

The carbon dioxide carbohydrate ratios will be still greater than those

calculated in Tables VI-VIII, if account is taken of the fact that some of the carbohydrate lost is not used in respiration, but in such processes of growth as the building up of the cellulose framework of the plant during development.

TABLE VIII.

The Carbon Dioxide Carbohydrate Ratio of the Continuous Aerobic and Anaerobic Respiration of 200 Rice Seedlings.

Stage of germination.	Anaerobic (dark).			Aerobic (dark).		
	CO ₂ (mg. in 24 hrs.).	Loss of carbohydrates as hexoses (mg.).	Ratio.	CO ₂ (mg. in 24 hrs.).	Loss of carbohydrates as hexoses (mg.).	Ratio.
1st-2nd day	80.6	40	2.01	144	132	1.09
2nd-3rd "	77.0	134	0.57	180	238	0.75
3rd-4th "	72.0	70	1.02	274	216	1.26
4th-5th "	68.6	65	1.05	319	134	2.38
5th-6th "	59.4	82	0.72	330	196	1.69
6th-7th "	74.2	95	0.73	340	114	1.99
7th-8th "	76.6	93	0.67	325	78	4.16
8th-9th "	59.2	55	1.07	257	163	1.57
9th-10th "	42.0	19.1	2.21	221	93	2.31
Total in 10 days	609	653	Mean 0.92	2390	1356	Mean 1.75

If the amount of carbohydrates (starch and sugars) disappearing is calculated from the actual loss in dry weight of the 200 seedlings which were dried and weighed each day the value is always less than the total loss of carbohydrates (starch and sugars) at each stage. The difference indicates that part of the carbohydrates disappearing which is used in the formation of cell-walls and protoplasm, i.e. for growth. The carbon dioxide carbohydrate ratios as given in Table VI, are 2.38 and 3.1 for the 200 seedlings germinated in air in the light and in the dark respectively will have to be increased considerably. This shows that in aerobic respiration the carbon-dioxide production is greatly in excess of the loss of carbohydrates (hexoses) in the germinating seedlings either in the dark or in the light. If similar corrections be made in the carbohydrates consumed in anaerobic respiration a similar conclusion, that carbon-dioxide production is in excess of the loss of carbohydrates as hexoses, according to the equation of anaerobic respiration, is arrived at. The carbon dioxide carbohydrate ratio for anaerobic respiration given in Table VIII is 0.92; actually it must be much higher, and calculations based on the difference between loss of actual dry weight and the total loss of metabolic carbohydrates raise this quantity to 1.72.

Estimation of Organic Acids and Alcohol in the Rice Seedlings Germinated in Air and in Nitrogen.

It is evident from the results obtained that at all stages carbon dioxide is given off by the germinating rice seeds in excess of that produced

by the respiration of hexoses. It was shown by Mayer (see 10) that in plant respiration oxalic acid and malic acid are formed in place of carbon dioxide, and the oxalic acid was considered by Warburg (see 10) and Puriewitch (see 10) as an intermediate product in the fermentation of sugars. But the modern view is that these plant acids are either produced by deamination of amino acids or from intermediate products of the synthesis of proteins during the growth of the seedlings. On account of the deficiency of nitrogen all the malic acid cannot be worked up into asparagine, and the excess of malic acid is oxidized to carbon dioxide and water. It is thus possible that the excess of carbon dioxide evolved by the germinating rice seeds comes from the oxidation of plant acids formed in connexion with the synthesis of proteins.

An attempt was made, therefore, to determine the acid contents of the germinating seedlings in air and in nitrogen. It is probable that under aerobic conditions the presence of the organic acids might not be detected, as they are likely to be oxidized as soon as produced, but under anaerobic conditions there should be some accumulation of these acids in the germinating seeds.

It is not possible to determine the organic acids individually, but the total acid was determined by the ordinary method of titration. The seedlings were germinated in a continuous stream of nitrogen for a period of ten days in the same manner in which they were germinated in nitrogen for the carbohydrate analysis. At every stage 200 seedlings contained in one of the galvanized iron pots were removed and killed immediately in a freezing mixture of ice and ordinary salt. After one hour they were removed from the freezing mixture and the juice extracted by first crushing them thoroughly in a porcelain mortar. The grinding and filtering was done repeatedly to ensure complete extraction of all the soluble matter from the seedlings. When finally filtered the total extract measured about 200–300 c.c.; it was preserved under toluene. 50 c.c. of the extract were titrated immediately against 0.0095 normal sodium hydroxide solution by adding four drops of the B.D.H. Universal Indicator. The concentration of the acid was then calculated in the usual way. In the control series the rice seeds were germinated in air and the sap extracted in the same way. It was found, however, that at each stage of germination the extract gave a neutral reaction, the concentration of organic acid was therefore taken as zero, with the Universal Indicator showing the total absence of plant acids in them. The results of the total acid contents in the anaerobic respiration of the seedlings is given below in Table IX.

In the absence of oxygen it appears that the organic acids are not oxidized, while in aerobic respiration there is complete absence of plant acids which are *probably* oxidized to carbon dioxide and water.

TABLE IX.

The Total Acids and Alcohol Contents in the Rice Seedlings Germinated in Nitrogen for Ten Days.

Stage of germination.	Normality of total acids.	Percentage of ethyl alcohol in gm.
1st day	0.0011	1.920
2nd "	0.0013	1.976
3rd "	0.0012	1.339
4th "	0.0018	1.508
5th "	0.0017	1.458
6th "	0.0014	1.394
7th "	0.0015	1.550
8th "	0.0014	1.550
9th "	0.0014	1.771
10th "	0.0013	1.4234

A striking point in this investigation is the complete absence of hexoses in the seedlings germinating in nitrogen (p. 63). This can be explained by the assumption that the hexoses are fermented as soon as they are produced. If they are fermented to carbon dioxide and alcohol it should be possible to estimate the alcohol in the seeds germinating in nitrogen. Using the water extracts of the seedlings and employing Nicloux's method, the extracts, clarified with basic lead acetate, usually reduced the dichromate solution to a large extent. This might easily be mistaken for an indication of alcohol, but with extracts of seedlings germinated in air was due entirely to the reducing sugars present in the solution. In the seedlings germinated in nitrogen, however, it has been already observed in the present investigation in Table V that reducing sugars are completely absent, and this facilitated the estimation of alcohol by the above method. The seedlings germinated in air contained no alcohol, as the reduction observed could be accounted for by the amount of hexose present. The seedlings germinated in nitrogen showed clearly the presence of alcohol (Table IX).

The extracts of both of the nitrogen and air series were tested for aldehyde content by the Schiff's reagent both before and after clarification; in both cases the result was negative throughout.

CONCLUSIONS.

From the carbon dioxide carbohydrate ratios for both aerobic and anaerobic respiration it is seen that the carbon dioxide produced is greater than can be accounted for by the loss of carbohydrates respired as hexoses. A surplus of carbon dioxide is obtained in all the determinations.

The excess of carbon dioxide found in both aerobic or anaerobic respiration, which is an important result of this investigation, introduces a

fresh difficulty in interpreting the chemical processes involved in respiration.

It is possible that the excess of carbon dioxide is the result of oxidation of plant acids produced in the synthesis of proteins. It is known that oxalic acid and malic acid are so produced, and these acids, with deficiency of nitrogen, are oxidized to carbon dioxide and water. Though the seeds germinating in air show no organic acids, a certain amount of such acid is present in the seeds in nitrogen at all stages of germination. It is probable that in air the acids are rapidly oxidized to carbon dioxide and water, while they accumulate in the absence of air.

The total absence of hexoses in anaerobic respiration over a continuous period of ten days in nitrogen led to the examination for alcohol of the seedlings at all stages of their germination in nitrogen. The results showed that alcohol is present in the seeds germinating in nitrogen, but entirely absent from the seeds germinating in air.

SUMMARY.

Aerobic and anaerobic respiration of the germinating seeds of rice (*Oryza sativa*) were followed to determine the relation between the output of carbon dioxide and the loss of carbohydrates. The I/N ratio (i.e. the ratio of the intensity of anaerobic respiration to the intensity of normal respiration) is found to be 0.66 in the case of daily change of material, and 0.33 with no change of material.

The analyses of carbohydrates of the seeds germinated in air show that the total sugars are in larger amounts at those stages at which the carbon-dioxide output is higher. In the seeds germinating in nitrogen no hexoses are found at any stage, and the cane-sugar is present in very small quantity.

The relationship between the carbon-dioxide output and the total loss of carbohydrates as hexoses is given as the carbon dioxide carbohydrate ratio. The calculated value of the ratio is 1.48 for aerobic respiration and 0.48 for anaerobic respiration, while the actual ratios obtained are 3.09 and 2.08 in the former and 0.53 and 0.90 in the second case. If corrections are made for the carbohydrates used for the purposes of growth, the carbon dioxide carbohydrate ratio is still higher. The results indicate that in both types of respiration there is a surplus of carbon dioxide which cannot be accounted for by the loss in carbohydrates, and is very probably not the product of respiration.

It is likely that the excess of carbon dioxide is derived from the oxidation of plant acids formed in the formation of proteins; this introduces an error in the measurements of respiration by the usual method of carbon-dioxide evolution.

Though hexoses were found to be absent in the seeds germinating in nitrogen, ethyl alcohol was present. It is likely that the production of hexoses is hindered in nitrogen, and any hexoses produced are utilized immediately.

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A Cytological Study of the Development and Germination of the Teliospores of *Hyalopsora aspidiotus* (Pk.) Magn.¹

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With fifty-four Figures in the Text.

INTRODUCTION.

HYALOPSORA ASPIDIOTUS is a well-known heteroecious rust belonging to the Pucciniastreae, a sub-tribe of the Melampsoraceae, with uredinia and telia occurring on *Phagopteris* (*Thelypteris*) *Dryopteris* and pycnia and aecia on *Abies balsamea*. From the standpoint of host relationships the Pucciniastreae are an interesting group. With the exception of the members of the genera *Pucciniastrum*, *Calyptospora*, and *Thecopsora*, all the species are associated with ferns and conifers, and this association with hosts of ancient lineage is generally considered to be evidence of antiquity and primitiveness (Moss 25, Faull 13). If, as Arthur (3) supposes, the more primitive rusts are to be looked for on the most ancient hosts, then a study of the rusts occurring on ferns and conifers might be expected to shed some light upon primitive characters.

The genera that comprise the Pucciniastreae possess teliospores which are intra-epidermal or sub-epidermal. In a previous paper (28) the development of the teliospores was described and particular attention directed toward those forms in which the teliospores are produced inside the epidermal cells, namely *Calyptospora*, *Thecopsora*, *Milesia*, and *Hyalopsora*. During the course of this investigation *H. aspidiotus* was found to be especially favourable for a more detailed study. This rust is one of the few long-cycled forms possessing a perennial diplont, the mycelium overwintering in the underground parts, and appearing year after year in the same localities. The teliospores form extensive patches in the young fronds

¹ The material in this paper was included in a thesis presented to the Graduate Faculty of the University of Toronto in June, 1933, in partial fulfilment of the requirement for the degree of Doctor of Philosophy.

and germinate without a resting period. In addition, the nuclei were relatively large and appeared to be favourable for cytological study. The emphasis in earlier work in the rusts has been placed upon the origin of the binucleate condition, and as Jackson (19, p. 26) has pointed out: 'Very little attention has been given to the accurate determination of whether or not the two nuclei fuse in the teliospore, or to a study of the nuclear history in connexion with the development of the basidium.' An investigation was therefore undertaken of the development and germination of the teliospores, particularly with reference to the fusion of the nuclei and the divisions in the promycelium.

MATERIAL AND METHODS.

From infected fronds which showed the desired stages, small pieces were removed, fixed and embedded in the usual way. Flemming's weak solution was found to be the most satisfactory fixative, and the triple stain was chiefly used with it. In studying fusion in the teliospore, and division in the promycelium, the cytoplasm often takes the stain so deeply that it is difficult to make out the details of the nucleus, and to offset this difficulty, Newton's iodine-gentian-violet stain was employed. The results obtained were very satisfactory, the chromatin staining a deep bluish-violet, and the cytoplasm remaining perfectly clear.

In order to study the early stages of development, infected plants were grown in the greenhouse. Since *Hyalopsora* is perennial, the mycelium overwintering in the root stocks, it was possible to bring in the plants in the fall, overwinter them, and plant them early in the spring. A number of infected plants were therefore marked during the growing season, and when the leaves had died down were dug up and brought into the laboratory. They were kept in a cold frame during the winter, and in the spring were potted and placed in the greenhouse for study and observation.

DEVELOPMENT OF THE TELIOSPORES.

When the young shoots had reached a height of 2-4 cm. a few were removed and placed in 95 per cent. alcohol. Freehand sections revealed the presence of abundant mycelium in the intercellular spaces and occasional sub-epidermal uredinia near the tip of the shoot. The young leaves continued their growth, and thirty days from the time of planting had reached a height of 9-12 cm. and were completely unfolded. The infected fronds were marked by conspicuous, light-coloured, intercostal areas which were most prominent on the under surface. Examination of these areas showed abundant mycelium throughout the large intercellular spaces of

the mesophyll, as well as a few young teliospores in the cells of the lower epidermis.

The mycelium spread rapidly through the fronds and a week later the infected areas had expanded, and the cells of the lower epidermis revealed the presence of the characteristic teliospores. The development of the teliospores within the epidermal cells was accompanied by a change in the external appearance, the infected areas becoming slightly darker in colour, and as the teliospores began to mature these areas assumed a pronounced orange tint.

Field studies were made at Lake Temagami in Northern Ontario. In this region the teliospores began to develop about June 1, and germinated much sooner than under greenhouse conditions. On June 2, 1932, most of the spores were found to be developing, and a few were already mature. Promycelia were present on many of the fronds by June 6. Germination continued through the next few days, until by June 11 practically all of the teliospores had germinated and produced basidiospores.

An abundant supply of moisture was found to be essential for germination, as Ashworth (4) has described for the teliospores of *Puccinia malvaccarum*. The woods usually have plenty of moisture at the time when the spores mature and germination follows immediately. It occasionally happens that the woods become very dry, and under these conditions germination may be delayed. Early stages of germination were rare in fixations made during the day. A series of fixations were accordingly undertaken throughout the night, portions of infected fronds which had previously been marked being fixed at intervals of two hours. Examination of this material revealed the fact that the greatest number of young promycelia were found in the fixation made at 2 a.m., when the moisture content of the air was high. This discovery proved very useful, because plants could be brought into the laboratory, placed in a moist chamber, and studied carefully from time to time. In every instance the plants from the moist chamber showed germination within a few hours, and after twenty-four to thirty-six hours the lower surface was covered with promycelia. On June 3 at 10.15 a.m. there was placed in the moist chamber an infected frond, the leaf of which was light grey in colour, indicating that the spores were just beginning to mature. Twenty-four hours later the fronds were examined, and the light grey areas had assumed a faint orange tint, characteristic of the mature condition. Seven hours later the spores began to germinate, young promycelia could be found in abundance, and germination continued for the next twenty-four hours until all the infected area was covered with a dense mat of promycelia.

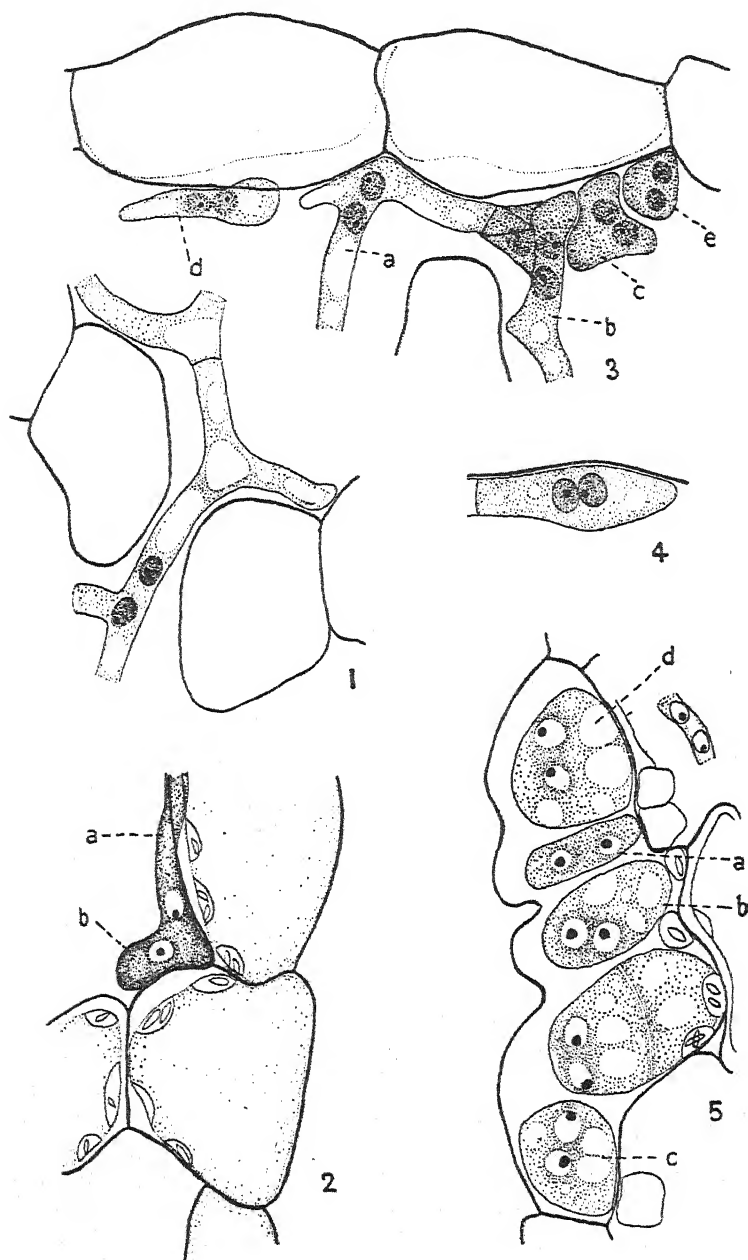
EARLY STAGES.

The earliest stages are found in the young shoot, where the mycelium, which has overwintered in the underground parts, is growing rapidly through the young tissues. The hyphal cells, in section, have a fairly regular outline where the intercellular spaces are large, but where the host cells are tightly packed the hyphae conform to the shape of the intercellular space. Haustoria are abundant, especially in the young cells of the curled tip. Later development of the mycelium is facilitated by the structure of the leaf, which is very simple, as Bower (7, Fig. 8, p. 8) has shown. The mesophyll is not differentiated into palisade and spongy parenchyma, but is composed of large irregular loosely-arranged thin-walled cells, with large air spaces, through which the mycelium continues to grow as the frond develops. The cells of the hyphae are binucleate, each nucleus containing a prominent nucleolus and being surrounded by cytoplasm in which large vacuoles are present (Fig. 1).

PRIMORDIAL CELLS.

The first indication of the beginning of primordial cell formation is the concentration of the mycelium just beneath the cells of the lower epidermis. Hyphae grow toward the epidermal cells and come to lie directly below. They may grow along just below the epidermal cells (Fig. 2 *a*), or they may grow at right angles to the leaf surface (Fig. 3 *a, b*). Where a single mycelial hypha gives rise to a primordial cell, the tip, which is closely applied to the epidermal cell-wall, begins to enlarge (Fig. 2 *b*), and then the nuclei pass in, the cytoplasm being very dense. This is the young primordial cell. A more advanced stage is shown in Fig. 3 *a*, where the hyphal strand is flattened out, and is growing along the cell-wall. As septations are formed each cell may become a primordial cell.

A number of hyphae in some cases may contribute to the formation of primordial cells, and as a result we have a compact group of irregular hyphal cells (Fig. 3). It often happens that where two mesophyll cells lie just below the epidermis, the hyphal strand must force its way between the two cells until it reaches the epidermis, and thus the tip becomes the primordial cell and is at right angles to the epidermis. This type of young primordium is well shown in Fig. 3 *b*. As the contents pass into the tip a septum is laid down cutting off a club-shaped cell (Figs. 3 *c, 4*), which forms the primordial cell. This cell is typically binucleate and the cytoplasmic contents stain very deeply. The hyphae below seem to be practically empty, and furnish a striking contrast to the well-filled primordial cells. There is a considerable variation in the shape and size of these cells. Where they are single they are usually club-shaped (Figs.



FIGS. 1-5. 1. Mycelium in mesophyll of leaf. $\times 1,150$. 2. Young primordial cell *b* formed by enlargement of the tip of the hypha *a*. $\times 1,150$. 3. Group of developing primordial cells in various stages. The hyphae *a* and *b* are growing at right angles to the leaf surface; *c*, *d* and *e* are primordial cells. $\times 1,150$. 4. A single mature primordial cell. $\times 1,150$. 5. Teliospore initials *a*, *b*, *c*, and *d* in the epidermal cell. Note the empty primordial cell below *a* and *c*. $\times 1,150$.

3 *d*, 4) and often reach a size of $15-18 \mu \times 3.9-5 \mu$, but where a number occur together they are smaller (Fig. 3 *e*) and more irregular (Fig. 3 *c*).

The number of primodial cells per epidermal cell varies greatly. A small epidermal cell may be subtended by just a single primordial cell (Fig. 2), although the number is usually much larger (Fig. 3), and a large epidermal cell may have from two to eight, or even more. There are usually five to six primordial cells to each cell of the lower epidermis.

The function of the primodial cells is to effect an entrance into the epidermal cells of the host, and to give rise to the intradermal teliospores. Their relation to the young teliospore is superficially similar to that of the haustorial mother-cell to the haustorium. In both the haustorial mother-cell and the primordial cell the host wall becomes pierced, and the contents pass in to form a small rounded or sac-like body, which is the haustorium in the case of the haustorial mother-cell, and the teliospore initial where the primordial cell is concerned. Beyond this, however, the resemblance does not go, since the haustorium is essentially a nutritive organ, making possible the growth of the organism, whereas the teliospore initial grows directly into a multicellular teliospore.

YOUNG TELIOSPORES.

The contents of the primordial cells now begin to pass into the epidermal cells. This passage of cell contents takes place very rapidly and it is extremely difficult to find the early stages. The youngest stage observed is shown in Fig. 5 *a*, where the empty primordial cell is clearly visible, while in the epidermal cell an elongated sac-like body may be seen. This is the teliospore initial, which by division gives rise to the multicellular teliospore. The young initials are somewhat spherical in outline, especially where they are developing singly (Fig. 5 *d*), but where a number of initials are growing in close proximity the shape is altered, and the initials tend to become elongated or narrow (Figs. 5 *a*, *b*). In a single epidermal cell the initials may show considerable variation in shape (Fig. 5). This depends entirely upon the size of the epidermal cell and the number of initials contained in it. The epidermal cell in Fig. 6 is very small in comparison with that of the previous figure and contains a single teliospore initial, illustrating the typical shape of the initials at this stage. The empty primordial cell, which has given rise to the initial, is shown below (Fig. 6 *a*). In the young initial the cytoplasm is very dense (Fig. 5 *a*) and the initial tends to stain very deeply, but as it enlarges the contents become distinctly vacuolate (Figs. 5 *b*, *d*, 6).

After the teliospore initial has enlarged considerably division takes place, and the two nuclei divide conjugately. The details of this nuclear division have not been followed, but it is clear that not only do the spindles lie side by side with their axes in the same plane, but the stages

in division in each figure are always identical. A transverse wall is laid down and the initial becomes two-celled; the two daughter nuclei in each cell are prominent and usually occupy a peripheral position near the end walls (Fig. 7). At this stage large vacuoles are conspicuous in the cytoplasm. A later two-celled stage is shown in Fig. 9, which shows a horizontal section through an epidermal cell, and indicates clearly the enlarged oval shape of the initial, and the central position of the two prominent nuclei. This two-celled initial is the developing teliospore, which conforms to the shape of the epidermal cell as further growth takes place. Growth and division continue until the epidermal cell is completely filled with the teliospores. The spores are divided by walls which are anticlinal to the surface of the leaf and which separate them into many cells, each of which is binucleate and well filled with distinctly vacuolate cytoplasm (Fig. 8).

FUSION NUCLEUS.

The two nuclei, which have been associated in the mycelium, the primordial cells and teliospore initials, now approach each other preparatory to fusion. The chromatin in each nucleus is in the form of very fine granules, the nucleolus is very prominent, and each nucleus is surrounded by a definite nuclear membrane (Fig. 9). The nuclei approach each other until they touch, and at the point of contact the membrane disappears (Fig. 10). The chromatin granules of each nucleus become aggregated at certain points, giving the appearance of coarser network. At first the outlines of the individual nuclei can be distinguished (Fig. 10), but as they fuse the outline is lost and the fusion nucleus becomes somewhat oval or oblong (Fig. 11). The nucleus now rounds up, the chromatin becoming finely granular and homogenous, while the two nucleoli remain quite distinct, and the whole nucleus passes into a resting stage.

The details of fusion in the rusts have been described by many authors, especially good descriptions having been given by Blackman (6) for *Phragmidium violaceum* and *Gymnosporangium clavariaeforme*, Moreau (23) for *Endophyllum Sempervivi*, Arnaud (2) for *Coleosporium Senecionis*, Colley (9) for *Cronartium ribicola*, and Allen (1) for *Puccinia malvacearum*; and in the lower basidiomycetes a clear account has been given by Gilbert (16) for *Dacrymyces*, and also by Fitzpatrick (15) for *Eocronartium*. In the descriptions given by these different authors there is a very close agreement in the stages that have been observed as the nuclei unite. The combined account of these authors could be applied almost in its entirety to the fusion of the nuclei as it has been observed in *Hyalopsora*.

Prophase.

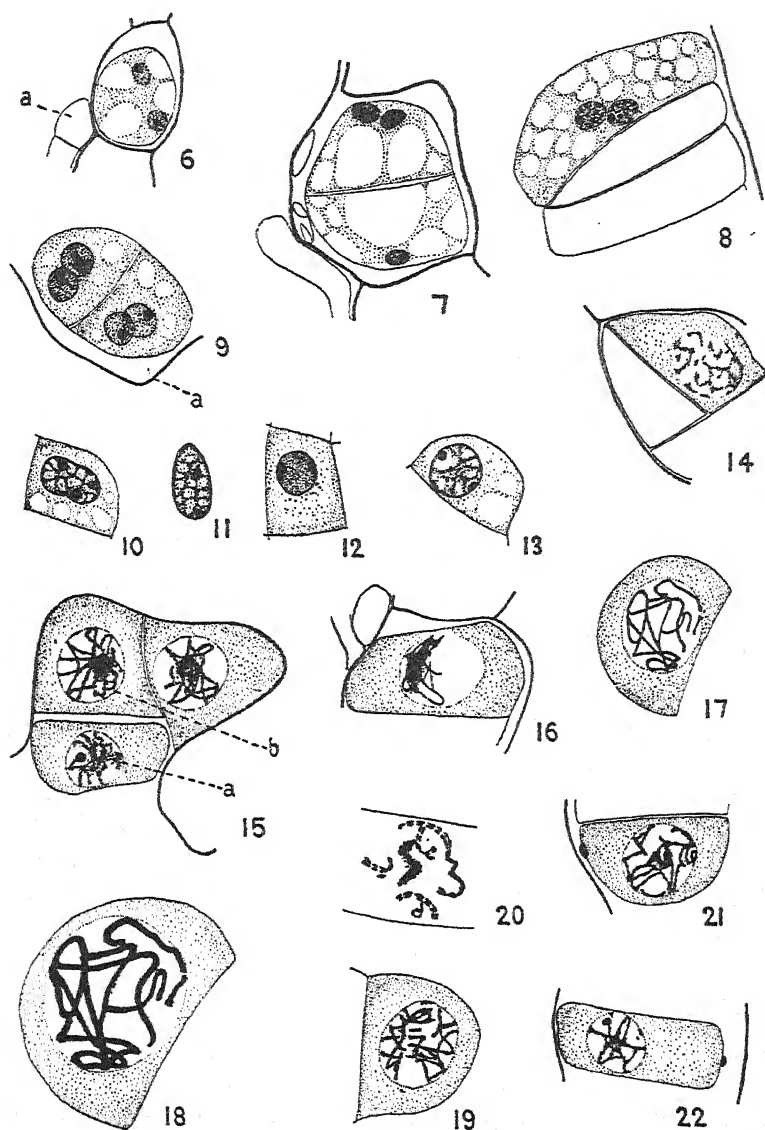
The fusion nucleus does not remain long in the resting stage, and almost immediately enters into the heterotypic prophase. The chromatin

becomes aggregated at certain points on the linin threads to form a coarse network, and the nucleus begins to increase in size (Fig. 13). It has somewhat the same appearance as the fusion nucleus in Fig. 11, but is readily distinguished by its larger size. The two nucleoli are still present and show no signs of fusing, although there is only a single nucleolus in the later stages. The fusion nucleus continues to increase in size until its diameter is two to three times that of the resting stage. The chromatin, which is in rather diffuse patches (Fig. 14), now forms an irregular spireme thread (Fig. 15 *a*), gradually becoming more even and looped (Fig. 15 *b*). At this stage a single nucleolus is present, intimately connected with the spireme threads, and often appearing to be the locus of a concentrated number of loops. So far I have found no suggestion whatsoever of polarization of these loops, nor is there any evidence of the presence of centrosomes. Occasionally the thread is found contracted into a dense heavily staining knot at one side of the nucleus, greatly resembling the bouquet stage of the higher plants (Fig. 16). Synapsis is thought to be represented by this so-called synaptic knot. Following this stage a well-defined thick spireme is formed (Fig. 17), and its characteristic appearance is shown under higher magnification in Fig. 18. Excellent differentiation can be obtained by the iodine-gentian-violet method, the chromatin taking the stain very deeply, and standing out in sharp relief against the colourless cytoplasm.

When material containing apparently mature teliospores is studied it is found that the majority of the nuclei are in some stage of spireme formation. Since the conditions necessary for germination may be delayed, the nuclei remain in this stage of prophase for a considerable period of time. The spireme appears to break up into a large number of pieces or segments, as shown in Fig. 19. In a good preparation the segments are seen to be double and composed of small paired units, the chromomeres, which give the thread a beaded appearance (Fig. 20).

In most plants meiotic prophase is followed immediately by spindle formation and the appearance of chromosomes. It was thought at first that in *Hyalospora* the actual division would take place in the teliospores, since all the stages of prophase were to be found there, but it soon became apparent that such was not the case. The division of the fusion nucleus has never at any time been observed in the teliospore, but always takes place in the promycelium. Ordinarily, germination follows as soon as the essential conditions are fulfilled, in this case an abundant supply of moisture being the primary requisite. At the time when the teliospores mature, there is usually an abundance of moisture, and germination is rarely delayed. However, it sometimes happens that under certain conditions, as, for instance, a dry spring, germination is delayed and the spireme stage is accordingly of long duration.

While the nucleus is in the spireme stage, a small, deeply staining,



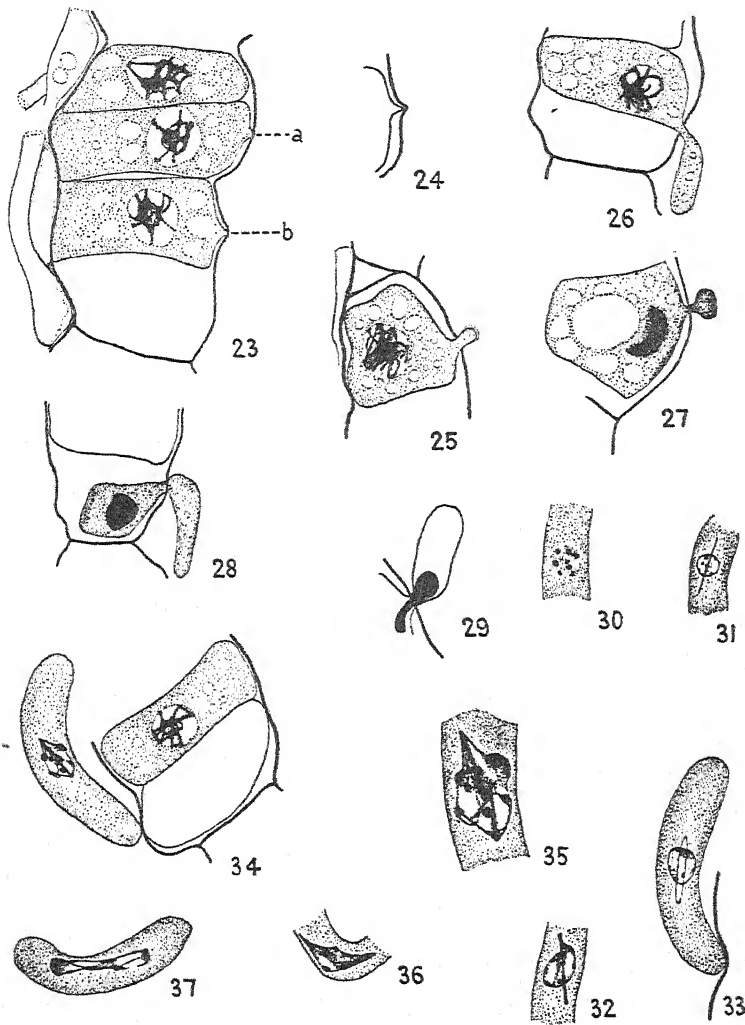
FIGS. 6-22. 6. A young teliospore with empty primordial cell *a*. $\times 1,150$. 7. Two-celled stage. Nuclear division has just been completed, the nuclei are in a peripheral position and cytoplasm has large vacuoles. $\times 1,150$. 8. Three cells of a later stage, one in detail. $\times 1,150$. 9. A two-celled teliospore. Host wall at *a*. $\times 1,150$. 10, 11. Fusion of the two nuclei. $\times 1,150$. 12. Mature fusion nucleus in resting condition. $\times 1,150$. 13. Early prophase. $\times 1,150$. 14. Later stage, nucleus much larger. $\times 1,150$. 15. Surface view of three cells showing stages in spireme formation at *a* and *b*. $\times 1,150$. 16. Synaptic knot. $\times 1,150$. 17. Later stage of spireme. $\times 1,150$. 18. Same. $\times 1,800$. 19. Fragmentation of the spireme. $\times 1,150$. 20. Paired chromomeres in the fragmented spireme. $\times 1,800$. 21, 22. Button-like body on outer wall of the teliospore. $\times 1,150$.

button-like body may be found on the outer wall of the teliospore. With the triple stain this body takes the safranin, staining a bright cherry-red colour, somewhat like the nucleolus, while with the iodine-gentian-violet stain it becomes a deep purple. In shape it is somewhat oval, or lens-shaped, often having the appearance of being flattened against the wall, and in size is about that of the nucleolus. This button-like body is intimately connected with the wall of the teliospore, being found embedded in the upper wall (Fig. 21) or more often seated directly on the wall, and standing out clearly (Fig. 22). Very little could be made out with regard to its structure, the whole body being very homogeneous and staining evenly throughout. In a few cases a slightly darker border has been found around the edge of the button, but these are of rare occurrence. Nothing is known of the origin of this button-like body, except that it appears on the wall during late prophase and disappears at the first indication of germination. In view of the fact that germ pores as such are absent in *Hyalospora*, it is of interest to know if there is any connexion between its disappearance and the germination of the teliospore. This point will be considered later in this paper.

DEVELOPMENT OF THE PROMYCELIUM.

The teliospore is now ready to germinate and the nucleus, having completed its prophase, is ready to pass into anaphase, which can take place only in the promycelium. The first indication of germination is found in the disappearance of the button-like body from the wall of the teliospore, and the appearance of a tiny papilla at the point where the button was previously found (Fig. 23 *a*). The papilla, which is very minute, is sharply pointed, and moves upward toward the overlying wall of the epidermal cell, raising it slightly at the point of contact (Fig. 23 *b*). The cell-wall is apparently flaccid and is readily pushed up by these sharply pointed papillae, so that the wall may be considerably stretched before penetration takes place (Fig. 24). As soon as penetration has been accomplished the cytoplasm commences to pass outward through the opening in the cell wall (Fig. 25). There is a suggestion here that the wall may have been altered at this point, part of the wall being carried upward by the protrusion of the cell contents, and tightly stretched before penetration is finally complete.

Coincident with the penetration of the host cell-wall and the beginning of the passage of the cell contents, is a change in the fusion nucleus. The segments lose their identity, forming a tangled mass, while the whole nucleus begins to contract (Fig. 25) and move toward the upper part of the cell (Fig. 26), meanwhile decreasing in size until it is about one-third that of the nucleus shown in Fig. 17. The condensation of the chromatin



FIGS. 23-37. 23. Germination. Papilla of spores at *a* and *b*. $\times 1,150$. 24. Papilla in contact with host cell-wall. $\times 1,150$. 25-28. Passage of contents into young promycelium. Nucleus contracts greatly. $\times 1,150$. 29. Nucleus passing in promycelium. $\times 1,150$. 30. Late prophase. Chromosomes inside nuclear membrane. $\times 1,150$. 31. Division figure showing rod-shaped spindle and chromosomes. $\times 1,150$. 32. Chromosomes moving on to spindle. Nuclear membrane persistent. $\times 1,150$. 33. Spindle fibres moving apart. $\times 1,150$. 34. Anaphase. Chromosomes cannot be differentiated. $\times 1,150$. 35. Same. $\times 1,800$. 36. Slightly later anaphase. $\times 1,150$. 37. Telophase. $\times 1,150$.

continues until the nucleus is contracted into a heavily staining body in which all details of chromatic structure are lost (Fig. 27). The nuclei in this stage have a characteristic staining reaction, retaining the stain strongly, even after all the other nuclei in the section have been completely destained. While this has been taking place the cytoplasm has begun to pass into the promycelium, and small vacuoles appear (Fig. 25) which increase in size (Fig. 27) until a certain point is reached, after which the remaining cytoplasm gathers around the nucleus and moves toward the neck of the promycelium (Fig. 28).

The nucleus, as it reaches the opening, is small, dark, and somewhat irregular (Fig. 28). During its passage through the neck of the promycelium, it becomes greatly attenuated, and slips through while in this form. As the nuclear mass emerges it tends to round up, assuming a characteristic pear-shaped appearance (Fig. 29). When this passage has been completed the nucleus rounds up and moves toward the middle of the promycelium. This sequence of events is similar to that given in the descriptions by Blackman (6), Dodge and Gaiser (12), and Gilbert (16). At the time when the nucleus enters, the promycelium has not attained its full size and the cytoplasm is very dense and homogeneous, staining very heavily at first. The entire contents of the teliospore cell pass into the promycelium, which increases in size, becoming somewhat curved and bending back toward the surface of the leaf, often touching it (Fig. 33). Although some variation has been found in the size of the mature promycelia, the majority are about 50μ in length, and 10μ in width.

Nuclear division in the promycelia.

Since the nucleus has already completed early stages of prophase in the teliospore it passes through the remaining phases with great rapidity. In the promycelium no stages of spireme have ever been found, the chromatin appearing inside the nuclear membrane as small deeply staining units which are undoubtedly the chromosomes (Fig. 30). A rather surprising feature of the nucleus in the promycelium is a marked reduction in volume. This is clearly shown when Figs. 23 and 30 are compared. Miss Allen (1) found in *P. malvacearum* that the nucleus, after migrating through the narrow neck of the promycelium, enlarged once more, and the chromatin 'loosened up' into delicate strands which shortened and thickened to form the chromosomes. In her figure of this stage the size of the nucleus, however, is much reduced. *H. aspidiotus* differs in that the nucleus is in a later stage of prophase when migration takes place, and chromosomes are formed as soon as the nucleus has passed out of the teliospore. It has not been possible to determine the number of chromosomes in these stages. Not only are the chromosomes extremely

small, but it is difficult to obtain a preparation in which they are sharply defined. Considerable variation has also been noted when chromosome counts have been attempted. In many cases only a few chromosomes have been found. At other times, however, the number of chromatic bodies is larger. Sass (29) reports that in *Coprinus ephemerus* the chromosomes appear one by one inside the nuclear membrane, and his figures for these stages are similar to those I find for *Hyalopsora*. This method of chromosome origin is highly improbable in the light of other evidence in the literature.

The early stages of spindle formation have not been found in the material so far studied. A typical spindle figure is rod-like or cylindrical with fairly broad ends, somewhat curved, and about two to three times the diameter of the nucleus. In polar view the median part of the spindle is seen to be in contact with the nuclear membrane, with the two ends projecting beyond the nucleus. When this stage is viewed from above, the rod-like spindle and the centrally placed nucleus give a characteristic rod-and-ring appearance to the whole figure (Fig. 31). From such a view it appears also as if the spindle might pass through the centre of the nucleus. That this is clearly not the case is shown when the spindle is studied in side view. The spindle passes near the surface of the nucleus, but whether it is actually embedded in the chromatin mass, or whether it is closely appressed to the outer wall with mantle fibres passing into the nuclear cavity, is a point which can be determined only by a further study of the origin of the achromatic structure.

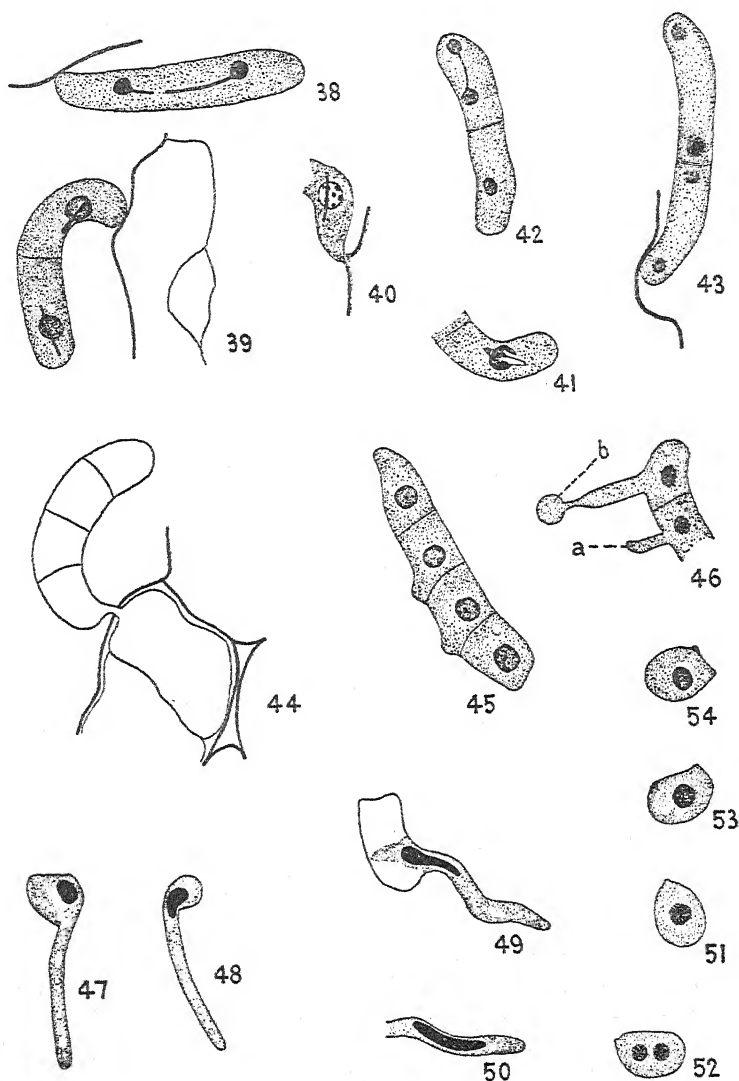
The spindle is clearly fibrillar in structure. When heavily stained or when differentiation has not been carried sufficiently far, the spindle appears as a solid rod. Bělář (5) found in *Aggregata eberthi*, where the spindle fibres were in the form of two 'achromatic cones', that when differentiation was not carried far enough some of the haematoxylin remained in the spindle, giving it the appearance of a solid rod. Fig. 32 suggests that from the poles of the spindle mantle fibres grow in toward the nuclear membrane which begins to disappear. In this figure the outline of the nucleus can still be made out, though the membrane has apparently disappeared at one point and the mantle fibres have become attached to the chromosomes. The connecting fibres gradually become less and less distinct (Fig. 33), as the mantle fibres grow into the nuclear cavity (Fig. 34). This figure, which is typical of this stage, is shown under higher magnification in Fig. 35, but it is impossible to recognize individual chromosomes, the chromatin being in irregular clumps.

I have never found so far any suggestion of an equatorial plate. The chromosomes are not sufficiently large or distinct to permit one to see the actual separation of daughter halves (Fig. 36). As anaphase commences the chromatin is drawn to the poles in a very irregular fashion, and the

spindle elongates and becomes much narrower (Fig. 37). It will be noted here that while the poles are rather broad there is no suggestion of conjugate polarity, nor is there any evidence that the spindle is double.

As the chromatin reaches the poles it becomes massed in two densely staining units, and a membrane is formed about each daughter nucleus. The connecting fibres become drawn into a single curved strand which later becomes broken (Fig. 38). A wall is now formed at the middle of the promycelium and the two daughter nuclei immediately prepare to divide. I have never found the nuclei in the so-called resting condition, which indicates either that prophase has been completed so rapidly that this stage has been missed entirely, or that the resting condition as such does not occur at this stage in the life cycle. It has been shown earlier that germination is very rapid, and therefore it is entirely possible that the daughter nuclei do not enter into a resting condition.

Inside the nuclear membrane chromosomes appear and a division figure is formed (Fig. 39). The spindles of the second division were found abundantly, and are typically of the rod-and-ring type as in the first division. Early stages of spindle formation have not been observed, and I have been unable to determine whether the spindle lies at one side of the nucleus or passes through the nuclear membrane. Occasionally in some promycelia spindles have been found lying free in the cytoplasm, and it would appear as if they have been disturbed by the microtome knife. It is obvious that the formation of the spindle and its relation to the chromosomes can only be understood by a careful study of the early stages of its development. Although smaller than those of the first division, the figures are very distinct and show clearly the chromosomes inside the nuclear membrane and a cylindrical curved spindle (Fig. 40). In a later stage mantle fibres are apparently present, although the details are not so clear (Fig. 41). Telophase figures are typical, with the connecting fibres in a single strand between the two daughter nuclei (Fig. 42). When division is complete the spindle disappears, and the nuclei take up their position at the opposite ends of each cell (Fig. 43). Cross-walls are formed dividing the promycelium into four cells, each with a single nucleus. A typical promycelium in outline is shown in Fig. 44, indicating its characteristic shape with the narrow neck passing through the opening in the epidermal cell-wall, and connecting the promycelium with the now empty cell of the teliospore. The cells of a single teliospore germinate individually and not necessarily all at the same time. It is not uncommon to find one cell of a spore much farther advanced than a contiguous cell. In some cases one cell may show a well-developed promycelium, while a neighbouring cell as yet shows no sign of germination. As each epidermal cell contains a number of teliospores which are themselves multicellular, the final result of germination is that the lower epidermis of



FIGS. 38-54. 38. Later telophase. Connecting fibres form a single strand. $\times 1,150$. 39-41. Stages in second division. $\times 1,150$. 42. Telophase. $\times 1,150$. 43. Daughter nuclei of second division. $\times 1,150$. 44. Typical four-celled promycelium. $\times 1,150$. 45. Beginning of sterigmata. $\times 1,150$. 46. Young sterigma *a* and young basidiospore *b*. $\times 950$. 47-50. Passage of nucleus through sterigmata. $\times 1,150$. 51. Mature basidiospore. $\times 1,150$. 52. Basidiospore with two nuclei. $\times 1,150$. 53, 54. Button-like body on basidiospore wall. $\times 1,150$.

the infected area of the leaf becomes covered with a dense mat of promycelia, giving the leaf a light, fuzzy, or woolly appearance.

Each cell of the promycelium gives rise to a slender sterigma which bears a basidiospore at the tip. The sterigmata appear first as slight protuberances on the wall, usually on the curved side away from the leaf surface (Fig. 45). The terminal cell may form its sterigma either at the very tip of the promycelium, as in Fig. 45, or laterally, as in Fig. 46, while in the remaining cells the sterigmata are regularly lateral. As the young sterigma (Fig. 46 *a*) continues to grow the development of the basidiospore begins at its tip (Fig. 46 *b*). The sterigma when mature is a delicate elongated body, well filled with cytoplasm (Fig. 47). The nucleus now proceeds to pass through the sterigma into the basidiospore. It moves to the opening, becoming slightly beaked as it commences to enter the narrow passage (Fig. 48), and more attenuated as it enters (Fig. 49), finally passing into the basidiospore as an elongate narrow heavily staining body (Fig. 50). In a prepared slide it is difficult to find the mature basidiospores attached to the sterigmata, as most of them separate easily and become detached through handling of the material.

The basidiospores are thin-walled and somewhat oval in shape, with a small papilla which indicates the point of attachment (Fig. 51). They are usually uninucleate, but occasionally a precocious division takes place, resulting in the basidiospore becoming binucleate (Fig. 52). The mature basidiospore often shows a small heavily staining body which is connected in some way with the cell-wall, appearing in certain cases to be embedded in it (Fig. 53), while in others it seems to be more superficial (Fig. 54). It is somewhat button-like or lens-shaped in structure, and is strikingly similar in appearance to the body that is found on the wall of the teliospore.

DISCUSSION.

Despite the fact that germ pores have never been reported for the teliospores of *Hyalospora*, the occurrence of the deeply staining body on the spore wall at the point where germination takes place suggests that this so-called button is concerned in some way with germination, which in this case follows immediately. It disappears at the first sign of the pushing out of the promycelium. Since it is at this particular point that the papilla arises and penetration takes place, it is possible that the button may be concerned in some way with a change in the host cell-wall at the point where penetration takes place. The wall of the epidermal cell is pushed up by the papilla and often appears to be considerably stretched. While the papilla appears to pierce the epidermis in a purely mechanical manner, it is quite possible that the wall may have been softened and carried upward by the papilla before the actual penetration occurs.

Along the edges of the hyphae of *Botryorhiza Hippocratae*, Olive (27), in his preparations with Flemming's triple stain, found conspicuous thickenings which are coloured red. He was not able, however, to discover their significance nor their mode of origin. When these thickenings are compared with the buttons on the teliospore wall of *Hyalopsora* the resemblance is seen to be remarkable. The agreement is very close in shape, size, position on the wall, and staining reaction. Their function might conceivably be associated with that of haustorial formation since this genus is characterized by abundant botryose haustoria.

The aeciospores of *Gymnosporangium myricatum* were found by Dodge (10) to possess germ plugs, which arise by a thickening of the middle wall at the point where the germ pore is to be formed. These thickenings showed a rather flattened or basin-like shape, and had a different staining reaction. The plug, in his Fig. 33, bears a striking resemblance to the button in *Hyalopsora*, especially with regard to its position on the cell-wall. There is a difference, however, in that the germ plugs are persistent, aiding in spore discharge, while the button disappears at the first sign of germination.

Faull (14) has described the teliospores of the closely related genus *Milesia* as possessing germ pores, one to each cell of the spore. The teliospores of *Hyalopsora* and *Milesia* are alike, not only in their morphology, but also in the manner of their development. They are both thin-walled multicellular spores, borne in the epidermal cells of their hosts, and germinate without a resting period. The presence of germ pores in *Milesia* would lead one to expect them in the teliospores of *Hyalopsora*. Notwithstanding the fact that germ pores have not been demonstrated there is little doubt but that the button is connected in some way with germination, and it may be that germ pores, as such, have disappeared in *Hyalopsora* and are represented only by the appearance of these button-like bodies at the time of germination.

The button-like body on the basidiospore wall is probably similar to the so-called button on the wall of the teliospore. Buller (8) has described the basidiospores of the rusts as possessing thin smooth colourless walls, having no pores whatever, and capable of sending out a germ tube anywhere on their surface. Of interest in this connexion is the work of Vokes (30) on *Coprinus atramentarius*. She found that hyaline bodies were formed on the wall of the basidium by contact with the fusion nucleus. Sterigmata were developed at these points, the hyaline bodies remained at the tip of each, and were carried upward to the apex of the growing spore wall. The basidiospores shown in her Figs. 45 and 46 are similar in appearance to those of *Hyalopsora*. The point of interest is that the hyaline bodies marked the points on the basidium where protrusion took place, and these hyaline bodies were later present on the basidiospore

wall, so that they probably mark the points where germination would take place. It is likely that the bodies on the basidiospore wall in *Hyalopsora* mark the points where protrusion of the germ tube occurs.

One of the most striking features in the development and germination of the teliospores is that which follows upon the fusion of the two nuclei. After a brief resting stage the fusion nucleus increases tremendously in size, and the chromatin forms a well-defined spireme. In a horizontal section through an infected frond just before germination these nuclei stand out prominently, especially when stained with iodine-gentian-violet.

The details of prophase, given by other workers in the rusts, vary somewhat in the sequence of the stages observed. Holden and Harper (18) considered that in *Coleosporium Sonchi-arvensis* (*C. Solidaginis*) synapsis took place in the resting stage following nuclear fusion. No synaptic knot was found to be present, and the chromatin threads were described as being very dense in the resting nucleus. As the fusion nucleus increased in size a short thick spireme was developed, which showed definite double-ness. Except for the absence of a synaptic knot, this account agrees fairly well with my findings for *H. aspidiotus*.

Blackman (6) found a similar train of events, with the exception of a second spireme, which followed the resting stage and segmented directly into chromosomes. Moreau (23) obtained a large number of prophase stages in *Coleosporium Senecionis*. She found that fine leptotene threads were formed, followed by zygotene, pachytene, strepsitene, and finally chromosomes, and many of her figures are similar to those I find in *Hyalopsora*. The Moreaus (24) in 1919 described and figured many stages of the reduction division in *Endophyllum Sempervivi*. After the two nuclei fuse a short resting period is followed by a spireme and a definite synaptic knot. It is interesting to note here, as in *Hyalopsora*, that these stages took place in the spore before migration of the fusion nucleus into the promycelium.

The situation which Colley (9) described for *Cronartium ribicola* is similar, except that a spireme stage was found to precede the resting fusion nucleus. In *P. arenariae* described by Lindfors (22) the prophases have been found entirely in the promycelium. In a recent paper on *P. malvacearum* Allen (1) reports that the early stages of prophase took place in the teliospore. No resting stage was observed after the two nuclei united, the fusion nucleus immediately entering the prophases. The prophases were not entirely completed in the spore; the nucleus was found to round up again after passage into the promycelium and the chromatin loosened up into delicate strands, after which the chromosomes appeared.

In the basidiomycetes other workers find a similar sequence of events. Fitzpatrick (15) in *Eocronartium* has described a situation which agrees in almost every detail with that in *Hyalopsora*. Following a resting period

a spireme thread was developed by the massing of the chromatin in the interstices of the network. A synaptic knot was formed, after which the thread showed definite double structure, and segmentation followed. In *Dacrymyces*, described by Gilbert (16), the situation was much the same as in *Hyalopsora*, except that no resting stage was evident. Vokes (30) observed a slightly different situation in *Coprinus atramentarius*. Following fusion the nucleus entered into a stage described as homogeneous, which probably represents a resting stage. Increase in size followed and a spireme was evolved which was found to be double.

During the last twenty years our knowledge of mitosis in the rusts has slowly but steadily increased. Colley, Lindfors, Olive, Moreau, and other workers have clearly shown that the nuclear divisions in the rusts are true mitotic divisions accompanied by centrosomes or their equivalents, true spindles and chromosomes. The number of the latter is still an unsettled point. Earlier workers, as Sappin-Trouffy, Maire, Moreau and others, considered that two chromosomes were regularly present, although the number is much higher according to Colley, Lindfors, and Allen. It is very difficult to determine the number in some rust species, as for example, in *Caecoma nitens*, where Dodge and Gaiser (12) were unable to make out individual chromosomes on the spindle. A similar situation has been found in *Hyalopsora*, despite the fact that the chromosomes can be recognized and counted at the end of prophase.

The nature and origin of the spindle is by no means clear. Olive (26) reports that the spindle was formed between two centrosomes, the product of a previous division which had moved around to opposite sides of the nuclear membrane. In the early stages he believed that the spindle was similar to Hermann's 'centralspindel'. Further development of the connecting fibres and the divergence of the poles resulted in an elongate, somewhat rod-like spindle figure. In the multinucleate cells from the base of the aecium in *P. Cirsii-lanceolati* Olive found small division figures with spindle fibres at the side of the chromatin mass and projecting into the cytoplasm, showing a remarkable resemblance to the spindle figure in *Hyalopsora*.

An assumed connexion between centrosomes and spindle formation has been noted by many workers in the rusts, including Blackman, Christman, Moreau, Colley, and Lindfors. In *P. malvacearum* Allen (1) found a small speck on the nuclear membrane and a tiny dark-stained area at the poles in division. As she has pointed out '... this in itself does not prove the existence of a centrosome...' and 'Until the supposed centrosome can be traced through all stages of nuclear division and its behaviour as a centrosome can be proved, it cannot be stated with certainty that a centrosome is present in *P. malvacearum*'. There is little evidence for the presence of centrosomes in *Hyalopsora*. It has already been pointed out

that during division there is no suggestion of a darker spot or point at the poles. Moreover, a careful study of nuclei in the mycelium, the teliospore, and the promycelium, has failed to throw any light on this question. The large size of the fusion nucleus makes it favourable for the observance of centrosomes if they are present, but to date they have never been seen. It would be very desirable, in this connexion, to know the details of the early stages of spindle formation. Are centrosomes, as such, present or absent in *Hyalospora*? What role do they play in the formation of this rod-like spindle? Does this type of division agree with that of Olive and therefore with that of the Hermann school, or does it resemble the divisions that Lauterborn has described for the diatoms? Is this situation in *Hyalospora* characteristic of these so-called primitive rusts? It is hoped that further studies upon *H. aspidiotus* and related species will furnish the answers to these questions.

Juel (20), in 1898, working on *Coleosporium Campanulae* described a division figure which is very similar to those in *H. aspidiotus*. He found that after a short prophase the fusion nucleus became fine grained, probably a resting phase, and in this chromatin mass a short, rod-like body appeared which stained like the nucleolus. This body stretched, became long-cylindrical or spindle form, occupying a longitudinal position and passing through the middle of the nucleus. His figures indicate clearly a rod-like spindle, with the chromatin at the middle still retaining the outline of the nucleus, resembling Figs. 31, 39, and 40 of *H. aspidiotus*.

Guilliermond (17) working on *Saccharomyces octosporus* described a thin elongated spindle. He found that the nuclear membrane persisted for a time after the chromosomes appeared and the spindle elongated, so that his figures at this stage resemble the spindles of *H. aspidiotus*. Wingard (31) found a similar situation in *Nematospora* in which an attenuated, somewhat rod-like spindle was the characteristic feature of the division figures.

Among the basidiomycetes division figures are occasionally found which resemble the rod-and-ring division figures of *Hyalospora*. Juel (21) in 1916 figured a number of these in *Craterellus*, *Cantherellus* and *Clavaria*. His figures included some fifty stages of metaphase and anaphase which fall into two types, regular mitotic divisions with definite chromosomes on the spindle, and a second type of spindle which resembles very closely those found in *H. aspidiotus*. Thirty-two of his figures come in the first group and eighteen in the second. Unfortunately Juel has not described these latter figures in detail nor drawn attention to them in any particular way. The spindles were rod-like and slender, the poles sometimes being pointed at the ends. At the middle of the spindle was a round body, the nucleus, the contents of which were darkly staining and usually homogeneous. The rod-and-ring appearance of these eighteen figures is most

striking. In an oblique view, as in his Fig. 73, the rod-shaped spindle can be seen to pass through the edge of the nucleus, reappearing on the other side. In *Hyalopsora* a heavily stained spindle has the same appearance. Juel did not give successive stages in these division figures, so that further details are lacking. In all cases these spindles appeared in the second and third divisions of the basidium, and were never found in the first division. The fact that this type of spindle was found in eighteen of his figures indicates that it must be a fairly common occurrence.

In an earlier paper (28) the question of the relationship of the Uredinales and the Auriculariales was considered. The evidence presented was in line with the view that the Auriculariales had been derived from the Uredinales, and it was suggested that they had arisen from some point on the rust line near *Uredinopsis*, *Milesia*, and *Hyalopsora*. It may be pointed out here that cytologically there are several features in the Auriculariales which may indicate a relationship to *Hyalopsora*. Juel (20), in 1898, studied the nuclear situation in the basidium of *Auricularia mesenterica*, and one of the features of his division figures was an elongated, rod-shaped spindle on which the chromosomes could readily be made out. Unfortunately, very few of the Auriculariales have been studied cytologically. Fitzpatrick (15) working on *Eocronartium* has described the details of nuclear fusion and the stages in division that follow. He found that after a short resting period the fusion nucleus passed quickly into prophase and a spireme was evolved which became shorter and thicker, finally segmenting, each segment showing a definite split. This series forms an almost perfect parallel with early prophase details of *Hyalopsora*. Gilbert (16) studied *Dacrymyces* from a cytological standpoint and found a similar situation, except that the resting stage was lacking. The paucity of cytological studies of the basidium of the Auriculariales and of the nuclear details in the promycelia of the Uredinales renders it difficult to study their relationships from a nuclear standpoint. There is no doubt but that the two orders are closely related, and cytological studies in both groups are highly desirable, and would probably throw considerable light upon their relationships.

SUMMARY.

1. The mycelium of *Hyalopsora aspidiotus* overwinters in the underground parts of *Thelypteris Dryopteris* and grows up with the young shoots in the spring, spreading through the intercellular spaces of the fronds as they unfold.
2. Primordial cells develop in the intercellular spaces immediately beneath the cells of the lower epidermis.
3. Teliospore initials are formed in the epidermal cells by the passage of the contents from the primordial cells. Each primordial cell gives rise

to a single teliospore initial, which divides to form a multicellular teliospore.

4. The two nuclei in each cell of the teliospore unite to form a single large fusion nucleus which, after a brief resting period, enters into prophase and the nucleus passes through various stages of spore formation. The nuclei of the mature spores remain in prophase until germination takes place.

5. Germination has been found to be dependent upon an abundant supply of moisture.

6. A promycelium is developed and the nucleus passes through the narrow neck to take up a central position.

7. The two divisions which follow are characterized by the appearance in late prophase of free chromosomes inside the nuclear membrane, and also by the slender rod-shaped spindle, which gives the figures a characteristic rod-and-ring appearance.

8. The promycelium is regularly four-celled. The basidiospores are uninucleate, or occasionally binucleate.

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The Receptive Hyphae of the Rust Fungi.

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With five Figures in the Text.

THE occurrence of emergent hyphae in the rust fungi, exactly comparable with the 'receptive' hyphae of present-day investigators, was reported by de Bary (11) as long ago as 1884 in his 'Comparative Morphology of Fungi, Mycetozoa and Bacteria'. In his consideration of the mechanism of sex in the Uredineae he wrote as follows: 'In young groups of aecidia, a phenomenon is observed not infrequently and without great difficulty which seems to be in favour of the supposition that there is an archicarp duly equipped for conception; short, obtuse hyphal branches project from some of the stomata like the tips of the trichogyne in *Polystigma* and may be traced here and there to a young perithecium. But the chief point, the continuity of such a possible trichogyne with the supposed archicarp on the one side and on the other the distinct relation to the spermatia, has not yet been shown; there is nothing in the phenomenon observed which compels us to speak of trichogynes, and not simply of branches of the mycelium which may as well grow outwards from a stoma as in an inward direction into an intercellular space.' The use of the word 'archicarp' here is in accordance with de Bary's definition of it as the commencement of a fructification, and it is assigned to no special type of structure.

Similar hyphae protruding through stomata were also recorded by Richards (16) in *Uromyces caladii* on *Peltandra*. 'Occasional hyphae were seen protruding out of the stomata, but they did not connect with any of the primordia and showed no evidence of specialisation.'

Klebahn (14), too, in his examination of many types of aecidia found wefts of hyphae in open stomata. These appeared to be swollen at the tips and in connexion with spermatia. He also spread spermatial nectar on the surface of the leaf, but as this did not seem to effect the formation of aecidia he concluded that the spermatia were not necessary for their development.

In 1904 Blackman (7) figured a second type of emergent hypha in *Phragmidium violaceum*, which was pushing up between two epidermal cells. It will be remembered that in this study of the aecidial primordia Blackman had found uninucleate cells lying side by side, each of which cut off a sterile cell at its tip. Assessing the importance of these cells Blackman wrote as follows: 'Its position above the fertile cell would suggest that it formerly acted as a receptive cell, pushing up between the epidermal cells as a trichogyne to which the sticky spermatia could be brought, for example, by insects. Some support is lent to such a view by the fact that occasionally cases are to be found in which the sterile cells do push up between the epidermal cells and swell out above, being merely covered by cuticle. If the development were pushed one stage further and the cuticle pierced a very effective receptive organ would be the result.'

From the period during which Blackman's results were published until 1927 it was generally assumed that the spermatia were functionless, their place as fertilizing agents being taken by cell fusions in the aecidial primordia. In that year, however, Craigie (8, 9) recorded the results of his investigations on *Puccinia helianthi*, *P. graminis* and certain other rusts from which it seemed evident that the spermatia played an essential part in aecidium formation. Craigie's work, however, was purely experimental and no evidence was adduced as to the actual method of functioning of the spermatia. The first suggestion that they fused with exposed hyphae functioning as trichogynes came from Andrus (5) in his account of the 'Mechanism of Sex in *Uromyces appendiculatus* and *Uromyces vignae*'. Here he described both types of hypha to which reference has been made, namely, those reaching the exterior surface by means of stomata and those pushing up between the epidermal cells. He considered them to be the tips of trichogynes which were 'much branched and highly septate organs having their terminus at the epidermis of the host leaf where they project through the stomata or between epidermal cells and fuse with spermatia transferred by insects, by hand, or by other agencies'. In view of this supposed function he suggested the name 'receptive' hyphae for them. It was noted further that the tips of these hyphae stained more readily than other parts of the mycelium and so suggested the presence of a 'receptive' spot which was compared to that of the oogonium of *Vaucheria*. Their time of occurrence, some eight to ten days after inoculation, when the aecidial primordia were in process of formation, was cited also in support of this function. The evidence of fusion of spermatia with such hyphae submitted by Andrus is, however, far from convincing and indicates the need for more extended study. Andrus did not find hyphae of this nature in the teleutosori.

That this conception of the receptive hyphae as functional trichogynes is not new may be seen from de Bary's remarks, and it is interesting to

contrast Andrus's interpretation with the former's cautious assessment of all available evidence. Andrus considers that the two-legged cells, usually held to be binucleate fusion cells of the aecidium, are present before fertilization and their morphology is interpreted as showing a stalk cell, an egg cell, and a cell which constitutes the basal cell of a multicellular trichogyne.

Allen examined *P. triticina* (2), *P. graminis* (1, 4), and *P. coronata* (3) for similar evidence. In the former rust she found that 'while spermogonia are developing, certain hyphae are growing into stomatal apertures or forcing a passage way between the epidermal cells of the leaf'. 'Since these hyphae reach the surface of the leaf and serve to receive the spermatial nuclei, the terms "receptive hyphae" or "emergent hyphae" have been applied to them.' She stressed the importance of the stomatal hyphae and noted that the number of these in any one infection varied inversely as the number of spermogonia present. These hyphae were short lived, and as each shrivelled and died another one took its place. 'It was not uncommon to find one to six hyphae in almost every stoma in the infected area. The actual entry of the spermatial nuclei into the receptive hyphae was not seen.' 'That nuclei do appear in the receptive hyphae often in considerable numbers after the application of spermatia to the surface of an infection is amply demonstrated.' She also found that 'upon being fertilized the multinucleate condition is found, first in the hyphae at the epidermis, and later at points more remote from the surfaces of the leaf'. The hyphae emerging between epidermal cells were considered as points of possible fertilization, but quite how this is effected is unsolved for 'so far as the microscope reveals, both the cuticle of the leaf and the walls of the hyphae themselves are intact' and again 'the entrance of the spermatial nucleus has not left a visible pore'.

Her investigations of *P. coronata* produced similar results and the additional evidence that the receptive hyphae may be present in an infection before the spermogonia are formed, and also that they may develop next to these organs and even as branches of the young paraphyses, indicating that there is no potential difference between these two structures. Considering the intercellular type of hypha she concluded; 'these hyphae seem to be unable either to pierce the cuticle mechanically or to dissolve it chemically, but are able to effect a separation between the cuticle and the inner layers of the epidermal wall, and to grow for considerable distances beneath the cuticle,' and yet later she considered that the spermatia scattered along the surface of the epidermis could enter at any point where the mycelium reached the surface. 'The extremely small spermatial nuclei passed through the wall of the epidermal cell into the hyphae.'

In a paper on the cytological study of heterothallism in *P. graminis* (1) published before Andrus's paper Allen did not report the presence of

emergent hyphae and suggested that the diplophase in this rust was initiated by fusions of spermatia with the paraphyses of the spermogonia. Recently, however, (4) she has re-examined her material and now recognizes two phases of development. Before the aecidial primordia are formed, in infections on young barberry leaves there are a few intercellular emergent hyphae accompanying the spermogonia at the upper surface of the leaf, but these hyphae soon become confined by the increase in thickness of the cuticle. There are very few stomatal hyphae at the lower surface of the leaf. In older leaves there are a few intercellular hyphae and an increase in number of stomatal ones. After aecidial primordia are initiated, and often after the most favourable time for fertilization, abundant hyphae may be found in the stomatal apertures. This is attributed to the fact that, the increase of mycelium in the tissues of the leaf causes a certain amount of tension and this stretches apart the guard cells. The stomata are thus opened and space is available into which the hyphae may grow. The possibility of fertilization by means of these hyphae is accepted. 'It is probable that fertilization can also occur by means of the stomatal hyphae at the lower surface, although this has not been observed.' Since the intercellular receptive hyphae are often dead or confined by the cuticle, Allen suggests that fertilization takes place through the spermogonia.

In all her papers Allen admits that it may be a positive aerotropic response that brings all emergent hyphae to the leaf surface, whether in stomata, between epidermal cells, or at dead epidermal cells.

In none of her investigations does she suggest the presence of a clearly defined sex apparatus in the aecidium.

Allen's views as to the function of these emergent hyphae thus appear to fluctuate. In *P. triticina* and *P. coronata* she considers the stomatal hyphae to be the most important agents of fertilization with the intercellular hyphae as an auxiliary means, whilst in *P. graminis* fertilization is considered to be chiefly effected by fusion of spermatia with spermogonial paraphyses, with the stomatal hyphae as an auxiliary means and with the intercellular hyphae as playing no role at all.

On the surface the observations of Andrus and Allen appear to agree, but two important differences should be noted. In the first place, Allen found that the formation of receptive hyphae could antedate the initiation of the aecidial primordium and even the spermogonium, whilst Andrus considers them to be an accompaniment of aecidial formation. And, secondly, there is the conflict of evidence as regards the relation of the intercellular hyphae to the cuticle of the host. Andrus makes no comment on this, and his figures do not show any evidence of cuticle over the tips of the hyphae. Allen, on the other hand, stresses the fact that these hyphae rarely reach the outside air. The rupture of the cuticle seems an important point in fertilization. If this cannot be achieved

by the receptive hypha itself it would seem to be a far more difficult task for a minute spermatium.

Recently Rice (15) has reported the presence of receptive hyphae in *P. sorghi*. 'From hyphal runners under the epidermis, particularly under the upper epidermis, short hyphae push out through the stomata. Sometimes as many as eight project through a single stoma.' And later 'they occur near the spermogonia in positions which I have described above, but they occur more abundantly on the under epidermis near the aecidia.' She gave no evidence of fusion between a spermatium and a hyphal tip, but occasionally found sub-stomatal hyphae that were binucleate. She also reported the presence of deeply staining 'spheres' showing nuclei. These occurred amongst the spermatia on the leaf surface and also in association with the spermatial hyphae, but she arrived at no explanation of these spheres.

It does seem clear, however, that from evidence yielded from infections (which on account of their sterility are deemed to be monosporidial in origin) that such infections produce spermogonia, emergent hyphae, and aecidial primordia. To effect the further development of these, there must be some interchanging of spermatia comparable to the cross pollination of an angiospermous flower.

Whilst handling spermogonial and aecidial material of various types of the rust fungi, the author was impressed by the frequent occurrence of these hyphae. At the moment the intention is to describe and figure their distribution and time of occurrence in relation to other spore forms, and the appreciation of their full significance will be left to a later paper.

It seemed essential to determine whether hyphae of this type accompanied other spore forms, viz., uredospores and teleutospores, and more especially the teleutostori of those short-cycled forms without spermogonia e.g. *P. malvacearum*.

Material of the following rusts was available. *Melampsora larici-caprearum*, *M. larici-populina*, *Melampsoridium betulinum*, *Phragmidium violaceum*, *Endophyllum sempervivi*, *Coleosporium tussilaginis* and *Puccinia malvacearum*.

Melampsoridium betulinum.

To understand the development of *Melampsoridium betulinum* in the leaf of *Larix europea* it is necessary to recall something of the leaf anatomy. The whole leaf surface is covered by a thin but firm cuticle, whilst the stomata are confined almost completely to a number of furrows, usually three on either side the mid-rib, this being visible only on the lower side of the leaf. As a result of infection by basidiospores, spermogonia are first formed. These occur on both surfaces of the leaf and are sub-cuticular in origin. Under experimental conditions they appeared six to seven days

after inoculation. Accompanying these there was a marked development of mycelium in the sub-stomatal spaces. This consists of hyphae which appear to be morphologically comparable to the receptive hyphae of the

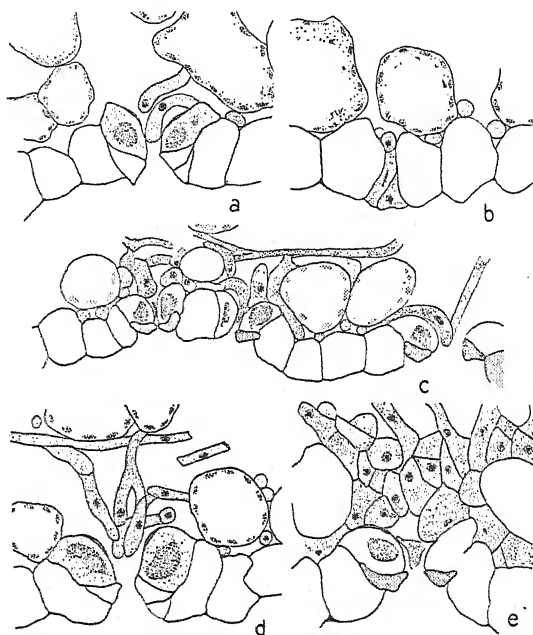


FIG. 1, *a-e*. *Melampsoridium betulinum*. *a*. Uninucleate hyphae pushing up between the guard cells of a stoma of *Larix europea*, eleven days after infection. *b*. Intercellular emergent hyphae. *c*. Massing of hyphae below stoma. Twelve days after infection. *d*. Several hyphae in sub-stomatal space. *e*. Sub-stomatal space filled with hyphae. Primordium of aecidium. (All $\times 1,120$.)

rusts studied by Allen and Andrus. Figures 1 *a* and 1 *c* give some idea of these. They are uninucleate and are often dilated at the tip. One quite distinct type is that which appears to grow round the guard cell of the stoma (Fig. 1, *c*). Several are usually seen making their way out to the stomatal opening (Fig. 1, *d*). The second type of emergent hypha is not so prevalent. This may be correlated with the presence of a firm cuticle. Sometimes cases like Fig. 1, *b* are seen, but here a second interpretation is possible, for this apparently intercellular hypha may be a part of the peripheral system of hyphae of the spermogonium.

One thing is certain, whatever the function of these emergent hyphae, a further development ensues leading up to the formation of the aecidium, for it is in this position that the aecidia are formed. A massing of hyphae takes place, the first stages of which can be seen in Fig. 1, *c*. The cells are uninucleate and remain so for some time, but they increase in number rapidly and pack the sub-stomatal space. Subsequently, hyphae are cut off at their tips whilst they are still uninucleate (Fig. 1, *e*). At this stage, nuclear migrations are sometimes seen.

In this case it is apparent that in ontogeny and position the stomatal hyphae are the precursors of the aecidia, but the question arises as to whether this development is controlled by something inherent in the fungus, or is merely a response to the utilization of the most favourable situation in the leaf tissue as regards space.

Occasionally, receptive hyphae were seen which appeared binucleate, but there was no evidence to suggest that this was the result of fusion with a spermatium.

In the uredosorus and teleutosorus of *M. betulinum* a few cases of hyphae pushing up between stomata were seen. One figured (Fig. 5, c) shows the tip of the teleutospore, still binucleate, passing between the guard cells of the stoma.

Melampsora larici-caprearum and *Melampsora larici-populina*.

The spermogonia and aecidia of these two rusts also occur on the leaves of *L. europea*, and can be conveniently described together. The development of the fungi in the leaf is very similar to that of *M. betulinum*. Spermogonia are formed under the cuticle on both sides of the leaf, whilst some hyphae grow towards, and eventually reach, the under surface and sub-stomatal spaces. These appear most frequently in material seven days old. The emergent hyphae appear as in Fig. 2 b with their tips between the guard cells of a stoma. Fig. 2 c illustrates a case in which the emergent hypha appears to have branched dichotomously. Intercellular emergent hyphae are frequent, but it is never possible to differentiate between these and the outermost hyphae of the spermogonia.

In material of greater age, a further development appears to take place at the stomatal openings, for the hyphae become massed as in *M. betulinum*. They are still uninucleate and undergo considerable development before the binucleate condition appears. Thus a similar series of events to that characterizing *M. betulinum* obtains.

Endophyllum sempervivi.

The presence of a perennating mycelium in this rust makes it impossible to state exactly the time of occurrence of these emergent hyphae, but it can be definitely stated that they are present as soon as the elongation of the diseased leaves is apparent. Examinations of material at this stage, shows that the greatest distribution of mycelium is in the sub-epidermal region of the leaf, and from here both the intercellular and stomatal hyphae make their way to the leaf surfaces. Macerations of moderately thin hand sections at this stage are often most useful, for the distribution of hyphae, and their frequent termination between the guard cells of the stomata can be seen well. Fig. 3 c was obtained in this way. Two hyphae can

be seen making their way between the guard cells, whilst they can both be traced back as branches of the same mycelium and in subsequent communication with branches which bear haustoria. In this case the emergent

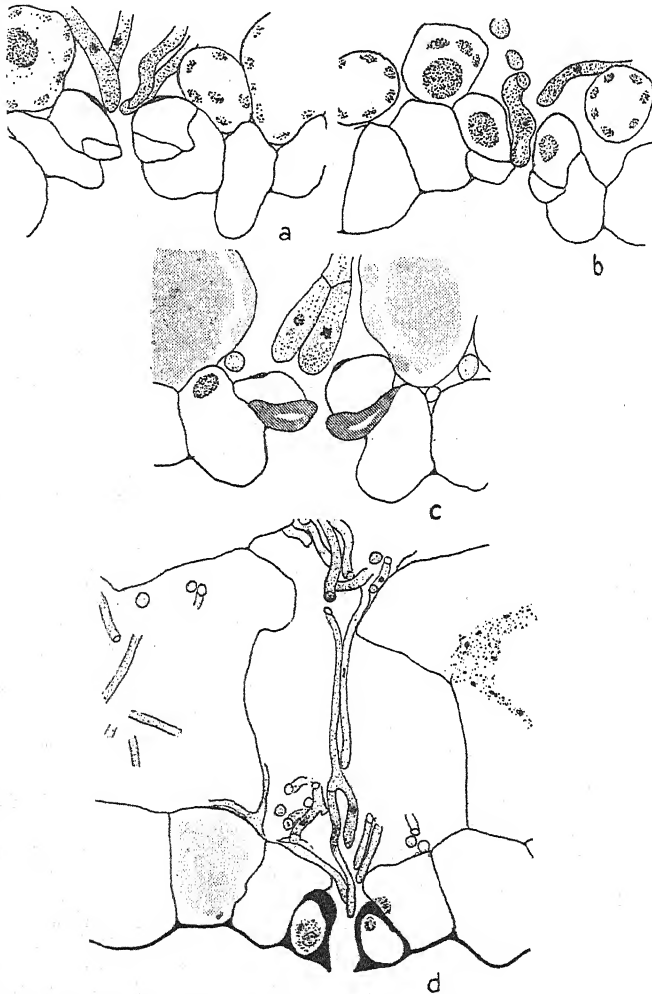


FIG. 2, a-d. a. *Melampsora larici-populina*. Hyphae in stoma. b. *M. larici-caprearum*. Hypha pushing between the guard cells of a stoma. c. *M. larici-populina*. Branched emergent hypha. d. *Endophyllum sempervivi*. Hyphae emerging between the guard cells of a stoma. (All $\times 1,120$.)

hyphae can be traced to a mycelium of a vegetative rather than of a reproductive nature.

But by far the greater number of hyphae of this nature seem to be the precursors of either aecidial or spermogonial primordia (Fig. 2 d), as may be seen from a consideration of the development of these sori.¹

¹ A detailed study of this rust will be given in a later paper.

Coleosporium tussilaginis.

The receptive hyphae of *Coleosporium tussilaginis*, have not been found in such great profusion as in the other rusts described, but this may be

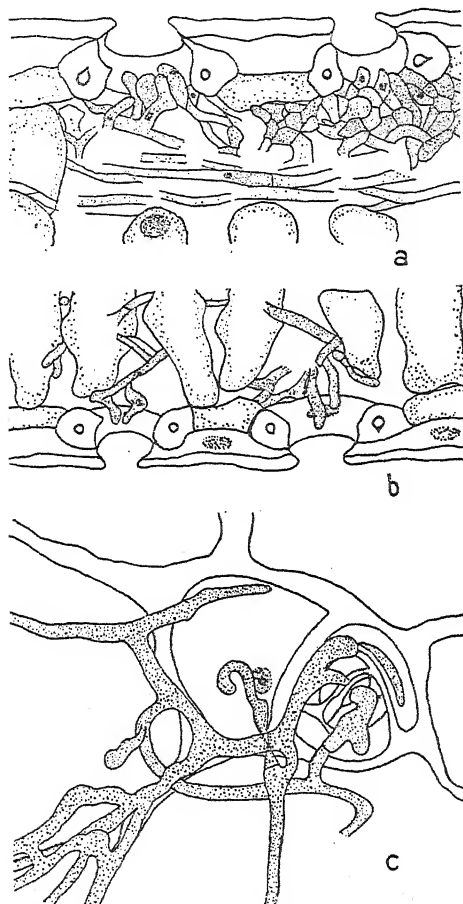


FIG. 3, a-c. a. *Coleosporium tussilaginis*. Hyphae in the youngest part of the aecidium grouping beneath stomata. $\times 450$. b. *C. tussilaginis*. Hyphae at the periphery of the spermogonium pushing up into stomata. $\times 450$. c. *Endophyllum sempervivi*. The mycelium in the sub-epidermal region of the leaf. Branches passing up between the guard cells before the initiation of spermogonia or aecidia. $\times 450$.

partly explained by the fact that it is more difficult to obtain material of exactly the right age, as the development of the rust is rather slow. The sporidia of *C. tussilaginis* infect the pine in October, November, and early December, and there the mycelium spreads until spermogonia appear at the end of March. In cultures indoors, in an unheated greenhouse, plants inoculated at the beginning of November produced spermogonia towards the end of January. On examination this material was found

to show a few examples of emergent hyphae. Fig. 3 *b* shows one of these. The stoma is cut longitudinally to the pore. In material a little older and in which aecidia are forming, similar cases were seen. Here a development similar to that in the larch rusts is evident. The aecidia are often some five or six millimetres in length, extending in the direction of the length of the leaf, and a median section through such a sorus usually shows certain cells in the centre which are binucleate, then a series of cells which are arranged at right angles to the epidermis, and finally a less regularly placed tissue in which certain of the hyphae protrude through the stomata. This is illustrated in Fig. 3 *a*. The stoma to the left shows the hyphae becoming arranged compactly at right angles to the surface of the leaf, whilst in that on the right the mycelium is not so compact in form, and a hypha can be seen protruding through into a sub-stomatal space.

Hyphae which push up between cells have not been found in this rust. The absence of these is no doubt conditioned by the particularly resistant cuticle.

Phragmidium violaceum.

The spermogonia and aecidia of this rust are of the indefinite type. It was found that spermogonia were first formed by hyphae pushing up between the cells of the upper epidermis. Such hyphae were most prevalent, and in view of the indefinite nature of the sorus, it was impossible to differentiate between hyphae destined to become spermatophores and others which might be considered receptive in nature (Fig. 4 *a*). As the hyphae spread to the lower surfaces of the leaf, the aecidial primordia were formed. Here hyphae very similar to those at the upper surface often protruded, but occasionally hyphae of a more active appearance were produced. In some cases these appeared to have broken through the cuticle (Fig. 4 *b*). There was also a considerable development of mycelium below stomata at the lower side of the leaf. For a long time these remained uninucleate, but later the cells were seen to have become binucleate. No receptive hyphae were seen in which there was more than one nucleus.

In the uredosorus, stomatal hyphae were of frequent occurrence and the further development of these caused massing of the hyphae in the sub-stomatal spaces and the eventual formation of uredospores.

Since this stage of the rust is produced by entry of the germ tube of the aecidiospore or uredospore through the stomata, such cases must be clearly distinguished from those described above.

Puccinia malvacearum.

A study of the occurrence and distribution of emergent hyphae in this form was thought to be of special interest in view of the short-cycled nature of the rust. No spermogonia occur, but it has been established that

the initiation of the diplophase can take place in cultures of monosporidial origin (6).

Examination of a considerable quantity of material of various ages revealed no development of emergent hyphae comparable with those

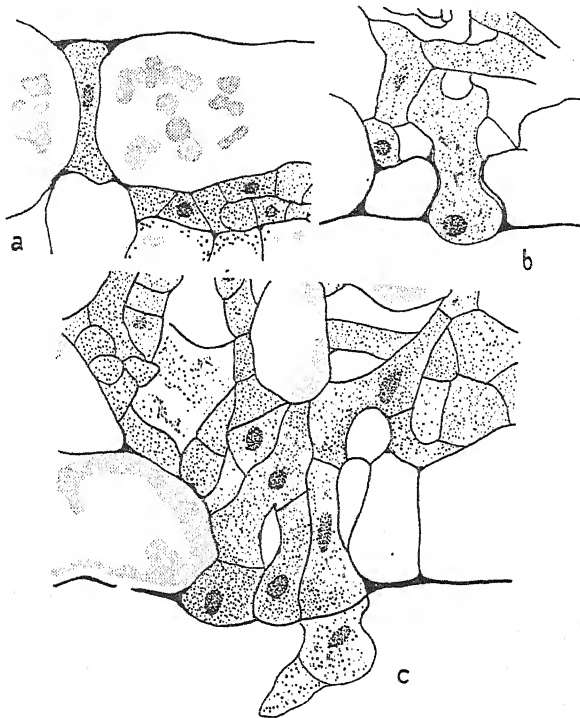


FIG. 4, a-c. a. *Phragmidium violaceum*. Intercellular hypha at the upper epidermis. b. *P. violaceum*. Intercellular hypha at the lower epidermis. c. Hyphae of *P. violaceum* in the region of an aecidium pushing through the lower epidermis. (All $\times 1,120$.)

described for the larch rusts above. The most marked feature of the sori at all stages was a good development of vegetative hyphae traversing the tissues of the host plant, and ending frequently in well developed haustoria. Those hyphae which ended in epidermal cells formed large haustoria. It has been noted elsewhere (6) that the onset of the diplophase seemed conditioned by the check to development received by these vegetative hyphae reaching the epidermis. Occasionally hyphae were found pushing up between stomatal guard cells (5 b, d). These seemed comparable with the neighbouring hyphae ending in the epidermal cells, differing only in that the intervention of a stoma in the epidermis provided a space through which the hyphae might grow. Sometimes too, there seemed to be a slight massing of hyphae in the sub-stomatal spaces. There was certainly no successive groupings of hyphae to be traced, as could be done in the larch rusts. The conclusion seems justified that in this rust there are hyphae

which occasionally reach the exterior of the leaf, but that they seem quite isolated from any subsequent development of the sorus.

The following table gives the distribution of the stomata at the epidermal surfaces, and the position of spermogonia and aecidia in the rusts under cultivation at Nottingham.

Rust.	Aecidial host.	Distribution of sperm. and aec.	Stomatal distribution.
<i>Endophyllum sempervivi</i>	Sempervivum	Scattered	Both surfaces
<i>Coleosporium tussilaginis</i>	Pinus	Scattered	Both surfaces
<i>Phragmidium violaceum</i>	Rubus	Sperm. upper; aec. under	Lower surface
<i>Melampsorium betulinum</i>	Larix	Sperm. both; aec. below	Lower surface
<i>Melampsora l-caprearum</i>	Larix	Sperm. both; aec. below	Lower surface
<i>Melampsora l-populina</i>	Larix	Sperm. both; aec. below	Lower surface
<i>Puccinia menthae</i>	Mentha	Scattered and on stems	Scattered
<i>Uromyces poae</i>	Ficaria	Scattered	Both sides
<i>Uromyces armeriae</i>	Armeria	Scattered	Both sides

The fact emerges clearly that on those leaves which possess stomata on the lower surface only, aecidia are present on that surface only, and that those in which the stomata are developed on both leaf surfaces have scattered aecidia. From this we might infer that the receptive hyphae do play some part in the formation of aecidia. In contradiction to this inference is Craigie's work (8, 9, 10) on *P. graminis* and *P. helianthi* in which he mixed the nectar on the upper side of the barberry and sunflower leaves—the non-stomatal surfaces—and yet got aecidia. If the anatomy of leaves is considered, two general types can be recognized, in the first place, those which possess a well-marked palisade tissue and in which the greater number of stomata and sub-stomatal spaces occur at the lower side, and consequently allow more space for fungal development, and secondly, leaves which possess no well-defined palisade layer, in which the stomata occur at both leaf surfaces and in which aecidia could be equally well developed at either surface. Further, the presence of a well-marked cuticle will tend to confine the fungal development to the stomatal areas. It is therefore suggested that soral development will be the greatest where there is most room available for it, and in most cases this will coincide with the sub-stomatal spaces. If this generalization is sound it ought to hold for the distribution of all spore forms. It was tested out in the following way. A list was made of all the rust fungi described in Grove's 'British Rust Fungi', together with the host plants and the distribution of the different spore forms, and then the position of the stomata was obtained from Solereder's 'Systematic Anatomy of the Dicotyledons' and Ward's 'Grasses'. An analysis of these data yielded several interesting facts:

(a) In general the distribution of soral forms and stomata coincided.

(b) In autoecious rusts the development of the aecidial, uredo and teleutosori occurred in the same position on the leaf, and this coincided with the greatest distribution of the stomata, e.g. *Phragmidium violaceum*,

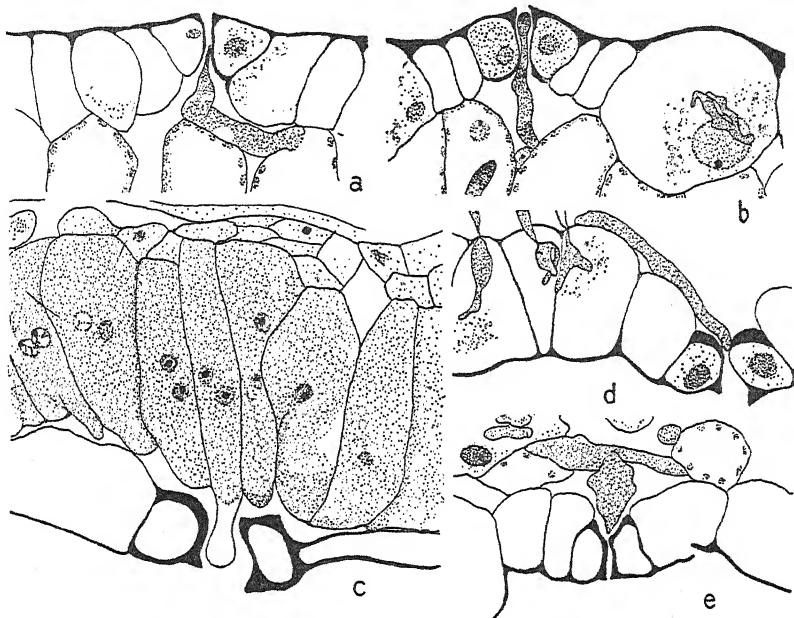


FIG. 5. *a-e.* *a. Puccinia malvacearum.* Hypha crushed between guard cells at the upper epidermis. *b. P. malvacearum.* Emergent hypha. Upper epidermis. *c. Melampsoridium betulinum.* Teleutosorus. Teleutospore crushed between the guard cells of the stoma. *d. Puccinia malvacearum.* Hypha emerging through stoma at lower epidermis. Note the well developed haustoria in the neighbouring cells. *e. Phragmidium violaceum.* Stomatal hypha at the lower epidermis of the uredosorus. (All $\times 1,120$.)

(c) The soral development of different rusts on the same host always occurs in the same position and is similarly related to the position of the stomata, e.g. all rusts of *Pinus* species have amphigenous aecidia, whether *Coleosporium* species or not, and the distribution of the stomata is amphigenous; the aecidial form of *Puccinia poarum* develops at the lower surface of the coltsfoot leaf, in a similar position to the uredo- and teleutosori of *C. tussilaginis*.

So it seems that the position of development of the sori can be explained from a consideration of the sub-stomatal spaces.

It is interesting in this connexion to recall the state of affairs reported by Dodge (12) in the systemic infection of the blackberry by *Gymnoconia interstitialis*. Normally, stomata are formed on the lower surface of the leaf only, but examination of infected leaves showed that a quarter to a half of the total stomata were developed on the upper surface. Further, they were formed in advance of aecidia, but whenever the rust at maturity covered only a part of the leaf surface it was found that, only that area

possessed additional stomata, showing that here again there is an association of stomata with aecidia.

SUMMARY.

1. Emergent hyphae of the stomatal type and the intercellular type are of frequent occurrence in the rust fungi examined.
 2. Their development is not confined to spermogonia and aecidia, but they may accompany other spore forms.
 3. Rusts without aecidia and spermogonia may produce emergent hyphae.
 4. It is suggested that the distribution of stomata and consequently the sub-stomatal spaces has a great influence on the position in which sori are developed.
 5. The presence of a thick cuticle is detrimental to the development of intercellular emergent hyphae and may suppress them altogether.
- In conclusion, I wish to express my thanks to Miss E. M. Blackwell and Professor H. S. Holden for their interest and helpful criticism.

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A Preliminary Study of the Water Loss of *Laminaria digitata* During Intertidal Exposure.

BY

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With one Graph in the Text.

THREE species of *Laminaria*—*L. saccharina*, *L. Cloustoni*, and *L. digitata*—are found growing at Port Eynon on the Gower coast, where the present work was carried out.

Both *L. saccharina* and *L. Cloustoni* live in deeper water than *L. digitata*, although an occasional specimen of *L. Cloustoni* may be found in deep pools within the *L. digitata* zone. At Port Eynon, large parts of the *L. digitata* zone are exposed by the diurnal tidal oscillations, which is not the case with the other two species. Judging from the tidal drift, *L. saccharina* is not as abundant as *L. Cloustoni*. Thus, among the species of *Laminaria* growing at Port Eynon, the conditions arising from an intertidal exposure affect *L. digitata* almost exclusively.

The *L. digitata* zone at Port Eynon is situated below the low-water mark of neap tides, and in the lower part of the zone *Laminaria* is the dominant and only large alga. It grows only in rocky situations, and is abundant in the deep pools about Skysea Point. The duration of intertidal exposure varies with the extent of the tidal oscillation, being greatest during the new moon spring tides. Also, individual plants situated in different parts of the *Laminaria* zone will be exposed for varying lengths of time. Some plants are barely exposed at all, even during spring tides, while few are ever exposed for longer than two and a half hours at a time. Although *L. digitata* may dominate over considerable areas of rocky substratum near low water, yet there is rarely a massing together of individuals to form dense communities, and thus 'mutuality' does not play a significant part as it undoubtedly does with *Ascophyllum nodosum*.

Methods of Investigation.

As soon as the tide had retreated from the upper limits of the *L. digitata* zone, twelve to twenty discs (1 sq. cm.) of newly-exposed seaweed

were punched out by means of a Ganong leaf cutter. The discs were then transferred into a clean, weighed and numbered tube stoppered with a waxed cork. Similar sets of sample discs were taken at regular intervals throughout the intertidal period, the same number of sample discs being adhered to throughout any single experiment. Each numbered tube with its contents was weighed as soon as it was convenient, and finally a graph of mass against time was plotted. This procedure proved excellent in the case of *L. digitata* with its smooth ribless frond, but, as will be seen later, there are seasonal variations in thickness to be taken into account.

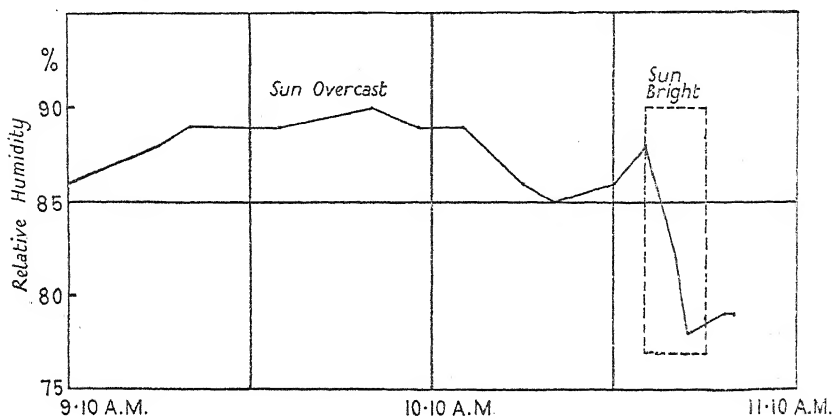
It was expected that the rate of water loss of *L. digitata* would be greatly affected by variation of environmental conditions, and so measures were made of relative humidity and 'sun' temperature. Humidity can be expressed as relative humidity or as a saturation deficit. An instance will make it clear that these two modes of expressing humidity are quite distinct. If the relative humidity of the air at 18° C. and 21° C. be 91 per cent., the corresponding saturation deficits will be 1.5 mm. (15.4–14.0) and 1.7 mm. (18.7–17.0) respectively.¹ The saturation deficit is the better index of the evaporating power of the air, for it gives us an absolute and not a relative deficiency which can be made good by evaporation.

In ecological investigations, however, the humidity of the air is most generally expressed in terms of relative humidity. In relation to the experiments carried out in 1927, the relative humidity of the air was measured by means of a wet and dry bulb thermometer. This instrument was deemed unsatisfactory as it was too clumsy to take out on to the beach, and consequently readings of relative humidity were not made at the reaction level of the plant. This also meant that it was not possible to make frequent readings of relative humidity. In the later experiments the 'Edney' Hair Hygrometer (Pastorelli & Rapkin, Ltd.) was used.

The relative humidity of the air is dependent on several factors, the most important of which is temperature. Winds also play a part. The temperature of the air and of solar radiation are not the same, but the latter will obviously greatly affect the former. The intensity of direct insolation may vary considerably during the day, and these fluctuations in solar radiation will bring about a marked and fairly rapid fluctuation in the relative humidity of the air. The relationship between these two factors will be most clearly seen on calm days when air currents are reduced to a minimum. The effect of variation in the intensity of solar radiation acting upon a particular spot was beautifully seen on February 18, 1928. Observations were made on the humidity of the air near low-water mark from 9.10 a.m. until 11 a.m. From 9.10 a.m. until 10.45 a.m. the sun remained entirely overcast by clouds, and there was no very marked

¹ The data used in this example were obtained from Landolt-Börnstein's 'Physikalisch-chemische Tabellen' (5).

fluctuation in relative humidity. At 10.45 a.m. the sun emerged bright and clear, and in a period of seven minutes the relative humidity fell from 88 per cent. to 78 per cent., the latter relative humidity being the lowest recorded during the entire period of observation. The effect of the sun



Graph showing variation in the relative humidity of the air near low-water mark.
Port Eynon, Feb. 18, 1928 (9.10 a.m. to 11.10 a.m.).

persisted for some time after it had again disappeared (see Graph). The above instance is only one of many observations showing a close correspondence between relative humidity and sun temperature, depressions in the radiation curve corresponding to rises in the relative humidity curve and vice versa.

Sun temperature was measured by means of a thermometer, the bulb of which was blackened and enclosed in a small corked glass tube. Since solar temperature and air temperature bear no fixed relationship to one another, it was not possible to correct by calibrating the thermometer used against a solar thermometer (or helioactinometer) which has its blackened bulb enclosed in a vacuum.¹

Experimental.

In Table I, intertidal losses in weight of *Laminaria* are given, together with figures of average relative humidity, standard deviations of relative humidity, sun temperature, and length of exposure.

During the summer months *L. digitata* is ideal material to work with, for then the thallus is of more or less uniform thickness. This is not the case in early spring (February–March), when the thallus consists of both new and old tissue. The old tissue is much torn, dark brown in colour, and bears large and elongated sori on its surface, while the new

¹ Further, the values given by different sun thermometers show marked differences depending on the degree of vacuum, size of bulb, amount and nature of the blacking material (2).

tissue—which is intercalated between the stipe and the old frond—is much lighter in colour. In some cases longitudinal slitting had made its appearance in the new intercalated frond tissue while the old frond was still attached.

TABLE I.
Water Loss in L. digitata.

Date.	Average relative humidity.	Standard deviation of relative humidity.	Average sun temp.	Time hours.	Final loss in weight
	%		° C.		%
1928.					
Feb. 18	85.5 [15] ¹	3.958	—	1.5	21.45
„ 19	76.0 [16]	2.634	11.3 [7]	2	22.7
„ 21	76.1 [12]	1.452	26.2 [12]	1.5	26.23
„ 23	82.3 [12]	2.758	14.5 [10]	2	19.31
1927.					
July 5	84.5	—	—	2.25	30.68
„ 1	89.0	—	—	1.0	8.3

On February 18 and 19, 1928, the sample discs were taken from the thallus as a whole, i.e. from both new and old frond tissue. In both cases there was a final loss in weight of about 20 per cent., but the curves obtained by plotting weight against length of exposure showed much greater irregularity than had previously been experienced. On further investigation it was found that discs of new tissue often weighed considerably more than twice the weight of discs of old tissue of equal area (1 sq. cm.). This, at least to a large extent, would explain the irregularities in the curves, for in any random set of discs the proportion of discs of old and new tissue would vary. Thus in subsequent experiments this source of error was eliminated by the expedient of taking discs of either old or new frond tissue, but not of both.

New thallus tissue studied alone gave very irregular curves, due to local variation in weight. It would seem that the new tissue was most compact and thickest nearest the stipe, while it got somewhat less thick and less compact towards its distal end.

With the old tissue it was quite otherwise, for, excepting the portions bearing sori, the thallus was of a uniform thickness. In every case a regular linear curve was obtained confirmatory of the results obtained the previous summer.

The data presented in Table I indicate that the loss of water from an exposed *Laminaria* is affected by the relative humidity of the air. The

¹ The figures in square brackets indicate the number of separate observations made.

period of exposure of the plants investigated on February 19 and February 23 was of the same duration, but as the table shows, the average relative humidity on the 19th was 76 per cent., while it was 82.3 per cent. on the 23rd, and the corresponding losses in weight were 22.7 per cent. and 19.3 per cent. From consideration of saturation deficit and the evaporating power of the air, it will be realized that were it not for the fact that the average sun temperature on the 23rd was higher than on the 19th the difference in the final loss in weight would be still more marked. The possible significance of average sun temperature and the importance of the distinction between relative humidity and saturation deficit is well illustrated by comparing the losses in weight on the 19th and 21st of February. On both days the average relative humidity was practically the same, but the loss in weight differed. Further, the discrepancy was greater than would appear from a consideration of final loss in weight alone, for the greater loss in weight was recorded during the shorter period of exposure; and also, the standard deviation of relative humidity calculated for that period was less than for the other. If, however, we compare the two periods of exposure in regard to average sun temperature, we find a marked difference. There can be no doubt, therefore, that although the relative humidity was the same in both cases, the evaporating power of the air was greater on February 21 than on February 19. On February 18, with a relative humidity of 85.5 per cent., a loss of 21.45 per cent. was recorded for 1 hr. 35 mins., while on the 23rd, with a relative humidity of 82.3 per cent. and an exposure period of 2 hrs. (5 mins.), the loss in weight amounted to only 19.3 per cent. The irregularity is at least to some extent, explained when we compare the standard deviations of relative humidity for the two dates. The standard deviation for February 18 was 3.96 per cent., while for the 23rd it was 2.76 per cent. Lastly, we may draw attention to the fact that the losses in weight for the 21st and 23rd clearly bear a relation to the relative humidity.

Losses of 8.3 per cent. for one hour and 30.7 per cent. for 2.25 hours were recorded in July, 1927. In the latter case there was a loss of 21.3 per cent. during the first hour of exposure. The difference in total loss in weight in the two cases was due to at least the following factors in addition to the difference in the duration of exposure.

(i) Different situations on the beach. The difference in period of exposure was a consequence of this. The *Laminarias* which were exposed for the longer period were situated in a more exposed position and were thus affected to a greater extent by such factors as wind.

(ii) A difference in the relative humidity of the air (see Table I), which in these experiments was measured by means of a wet and dry bulb thermometer. Further, there was some rain during the period of exposure on July 1, when the loss in weight at the end of an hour was only 8.3 per

cent. The loss of 30.7 per cent. sustained on July 5 certainly seems high, and is to be regarded as an index of possible drought resistance rather than an average loss in weight. It should also be remembered that this result was obtained in July, while the 1928 results were obtained during February.

The curve of water loss in *Laminaria* (total weight against time) is linear, and the loss in weight is fairly regular throughout, with a tendency to a somewhat greater loss at the beginning of a period of exposure than towards its end.

As already pointed out, many individual *Laminarias* undergo only a slight loss in weight during intertidal exposure, for they may be partly or wholly immersed in pot-holes or pools or situated in damp and deep clefts in the rocks. Thus on February 20, 1928, plants exposed for 1 hr. 5 mins. on very flat rock ledges near low water showed a loss of only 2.24 per cent. This, however, was an exceptionally low loss.

The data given in the present paper for *L. digitata*, and those previously presented for *Pelvetia canaliculata* (3), indicate that the littoral zonation of the larger Phaeophyceae may be to some extent an expression of the degree of water loss which the various species are able to endure during intertidal exposure.

The location of the reproductive organs also seems to be a factor bringing about zonation. The unilocular sporangia of *L. digitata* are massed together into sori on the surface of the thallus, and thus the vitality of the zoospores might be impaired by a considerable loss of water from the plant. Members of the Fucaceae, however, have the antheridia and oogonia sunk in conceptacles which open on to the surface of the thallus by means of an ostiole, and a certain amount of drying is necessary to bring about sufficient contraction of the thallus to force out a mucilaginous matrix containing the gametes so that fertilization may take place when the plants are again covered by the sea. Further, in the case of *Pelvetia*, the two functional oospheres are retained within the thick gelatinous wall of the oogonium.

A comparison was made of the water loss in *Fucus serratus* and *L. digitata* growing at the same level of the beach, the method of investigation being the same for both species, and in both cases a curve of the linear type was obtained. The loss in weight during the early stages of exposure was greater in the case of *L. digitata* than in *F. serratus*. The total loss in weight of the former was 19.3 per cent., while the total loss in weight of the latter was 16.9 per cent. (Two-hour exposure period.)

The intertidal losses in weight obtained for *F. serratus* are given in Table II below.

The procedure adopted can be considered as being fairly satisfactory in the case of *F. serratus*, for although this plant has a definite midrib there

is a fair amount of uniform tissue on either side, and also the midrib is not very much thicker than the other parts of the thallus. At the same time it should be borne in mind that the results obtained for *F. serratus* cannot, perhaps, be considered as reliable as those obtained for *Laminaria*, and this disparity is further exhibited by the slight wrinkling of the *F. serratus* thallus after long exposure. Owing to the slighter character of the thallus, larger numbers of sample discs were taken.

TABLE II.
Water Loss in F. serratus.

Date.	Average relative humidity. %	Standard deviation of relative humidity.	Time hours.	Final loss in weight. %
2.7.27	—	—	7	41.18
23.2.28	—	—	2	16.9
11.4.28	69 [9]	8.37	5.6	30.97

Table III gives the loss in weight at the end of a two-hour period for *F. serratus*, together with the final losses in weight of *F. serratus* and *L. digitata*.

TABLE III.

<i>Fucus serratus.</i>		<i>Laminaria digitata.</i>
Loss in weight at end of two hours.	Final loss in weight.	Final loss in weight.
16.5 %	41.18 % (7 hrs.)	22.7 % } 2 hrs.
16.9 %	16.9 % (2 hrs.)	19.31 % }
11.6 %	30.97 % (5.6 hrs.)	30.68 % (2.25 hrs.)
		21.45 % } 1.5 hrs.
		26.23 % }

Except in the case of the experiment carried out on February 23, 1928, it is not legitimate to make individual comparisons between the recorded losses in weight of *F. serratus* and *L. digitata* for periods of exposure of about two hours duration, since we have already seen in discussing the recorded final losses in weight of *Laminaria* that the loss in weight is greatly affected by the evaporating power of the air during exposure. We may, however, call attention to the fact that the final loss in weight of 41.18 per cent. for *F. serratus* and of 30.68 per cent. for *L. digitata* were both recorded within a few days of one another in July, 1927.

In the cases recorded, the losses in weight of *F. serratus* for a two-hour period are less than those recorded for *L. digitata*, but the figures (Table III) suggest that the former is able to tolerate greater final losses in weight

than the latter. As was pointed out earlier, the loss of 30.68 per cent. in 2.25 hours sustained by *L. digitata* is to be regarded as a somewhat extreme value for intertidal loss in weight for this species growing at Port Eynon.

Working at White Cliff Bay, near Bembridge, on the south-east coast of the Isle of Wight, Baker (1) showed that *F. serratus* had the maximum vertical range among the Phaeophyceae. This is also the case at Port Eynon, and thus the water loss during intertidal exposure probably varies to a greater extent than in other seaweeds. While, however, at White Cliff Bay, Baker found that the upshore extension of *Laminaria*¹ was nine inches below the maximum downshore extension of *F. serratus*,² at Port Eynon *F. serratus*, although not abundant, ranges from the lower reaches of the *Ascophyllum* zone to the upper ranges of the *Laminaria* zone, and thus at a certain level of the beach we find both species growing together. This is also true of the southern end of the Isle of Man.³

F. serratus may be restrained at the upper limits of the *Laminaria* zone at Port Eynon, because below that level the minimum of contraction necessary to bring about the liberation of gametes may not be possible. On the other hand, an exposure of the length borne by *Pelvetia* might be impossible to *F. serratus* without serious injury to the gametes, since the eight oospheres of *F. serratus* are extruded from the oogonium, while in the case of *Pelvetia* the wall of the oogonium persists around the two functional oospheres.

The writer wishes to emphasize the consideration that the data presented in this paper form only a preliminary examination of the intertidal drying of *L. digitata* and also of *F. serratus*. In the case of algae growing in the lower reaches of the littoral region and thus subject to relatively short intertidal periods, the evaluation of the degree of water loss is more difficult than in the case of algae growing near the high-water mark of spring tides. The final loss in weight in the latter case (e.g. *Pelvetia*) is not affected by the evaporating power of the air to such a marked extent as is the case with algae exposed for short intertidal periods. Thus an investigation of the water loss of such algae as *Laminaria* necessitates the carrying out of experiments under different weather conditions and at different periods of the year. In the case of *F. serratus* the fact of its wide vertical range has also to be taken into consideration.

I wish in conclusion to thank Professor R. C. McLean for the help which he gave me during the course of this work, and also Professor R. S. Adamson for his helpful criticisms of the manuscript of this paper.

¹ Dr. Baker did not state the species of *Laminaria* found at White Cliff Bay, but it would appear from the context that more than one species was present (1).

² Ibid. From data given in table (p. 197) and diagram (p. 198).

³ Knight, M. and Parke, M. W. (4) Plate IV.

SUMMARY.

1. Of the species of *Laminaria* growing at Port Eynon, the problem of water loss during intertidal exposure affects *L. digitata* almost exclusively.

2. The length of the exposure period of *L. digitata* varies with the tides and also with situation in different parts of the *Laminaria* zone. Some individual plants are hardly exposed at all, while few are exposed for longer than two and a half hours at a time.

3. Losses of 19.3 per cent. to 30.7 per cent. were recorded for periods of 2 to 2.25 hours.

4. Considerable variations in water loss are shown in relation to variations in relative humidity and sun temperature.

5. The superficial position of the unilocular sporangia of *L. digitata* may be a factor in preventing colonization of the higher ranges of the littoral region.

6. Comparison of the intertidal loss in weight of *L. digitata* and of *F. serratus* indicates that the latter species is able to endure a greater total loss in weight than the former.

7. *F. serratus* may be checked at the upper limits of the *Laminaria* zone at Port Eynon, as below this level the thallus may be unable to contract sufficiently on exposure to force out the gametes on to the surface of the plant.

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Meiotic Chromosome Structure in *Trillium erectum* L.

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With Plates I-III and eight Figures in the Text.

THE chromosomes of *Trillium erectum* L. are among the largest known in any organism. The shortest is 15μ long, and the longest 22.5μ , and their diameter, including the matrix, that is, as ordinarily stained, is about 1.4μ at the first pollen-grain metaphase (Text-fig. 1). In root-tip cells they are considerably longer. The complement normally comprises five pairs, each distinguishable by its size or by the position of the 'attachment constriction'. With appropriate methods of fixing and staining, the internal structure of the chromosomes is very clear during the later stages of meiosis; the chromonemata can be followed throughout their length. It is very favourable material for observing the origin and subsequent fate of chiasmata and for obtaining data relevant to theories of spiralization, chromosome pairing, and the mechanism of meiosis and crossing-over.

MATERIAL AND METHODS.

Corms of *T. erectum* were collected during September, October, and November of 1931, 1932, and 1933 from Ile Perrot, and Ste Agathe, Quebec. Early prophase stages of pollen mother-cell meiosis were in progress at the time of collection in most of the November material. When kept in a cool greenhouse the metaphase of the heterotypic division is usually reached towards the end of December; the homotypic division follows immediately, and the first pollen-grain division is completed early in January. Material collected on Ile Perrot is earlier than that from Ste Agathe, and that collected from the latter was more variable in 1933 than in either of the preceding years, possibly due to its having been collected earlier.

The pollen mother-cells were smeared and fixed in 2B, 2BD, 2BE (LaCour 1931), S_1 , S_2 (Smith (50), previously unpublished), Navashin, Bouin, Carothers' Bouin, medium-strength Flemming's solution, and various modifications of these fixatives. Bouin, 2B, and S_1 proved best for early prophases, while 2BD and S_2 were far superior to the others for revealing internal chromonema structure at the later stages. Fixatives containing urea mask all internal structure. Feulgen's method likewise gave no differentiation between the chromonemata and their matrix. The formulae of S_1 and S_2 are:

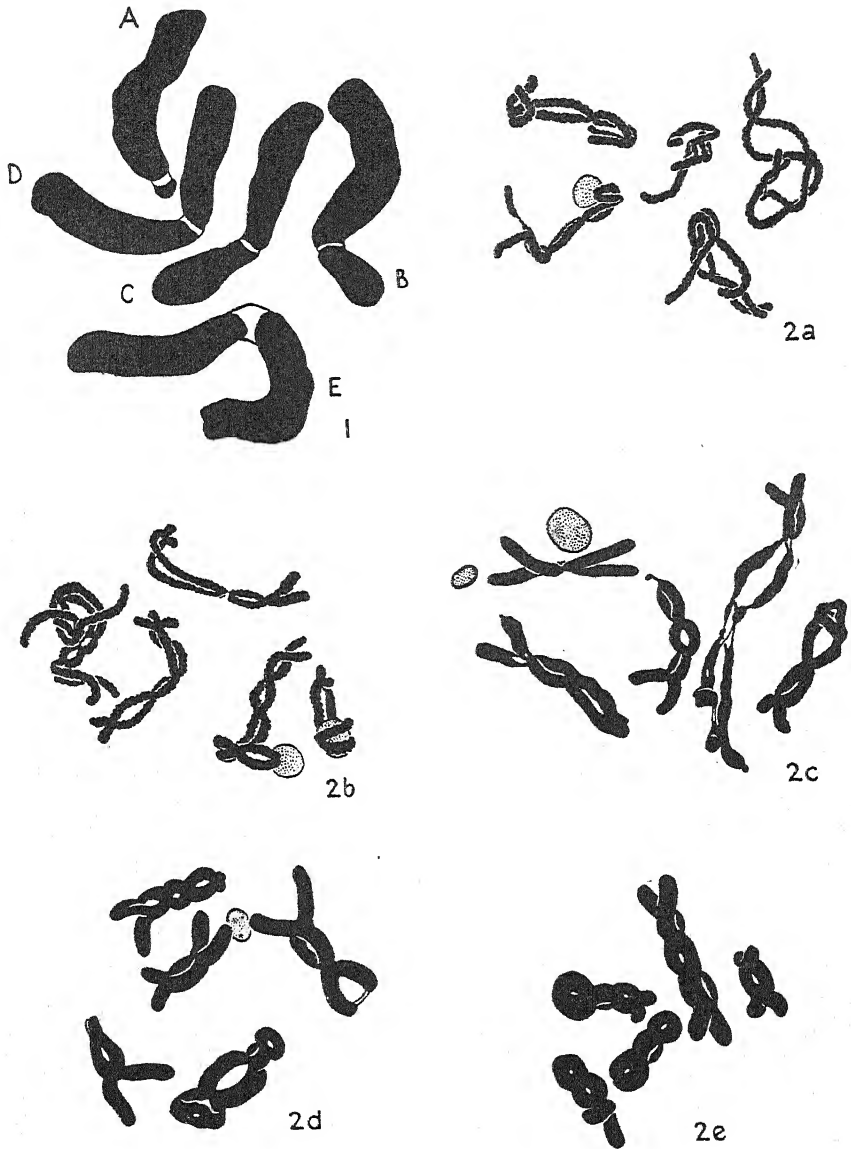
	S_1	S_2
Chromic acid 1 per cent. aqueous solution	100 c.c.	75 c.c.
Osmic acid 2 per cent. " "	30 c.c.	25 c.c.
Acetic acid 5 per cent. " "	30 c.c.	12.5 c.c.
Potassium bichromate . . .	1 gm.	1 gm.
Saponine 	0.1 gm.	0.05 gm.
Distilled water 	100 c.c.	46 c.c.

Crystal-violet staining was used for all the meiotic preparations here illustrated. Desiccation for 10, 20, and 30 seconds after smearing and before fixing was found greatly to increase the clarity of the spiral structure. After washing in water or alcohol, according to the fixative used, and after bleaching in H_2O_2 in 70 per cent. alcohol for 1-2 hours if the fixative contained osmic acid, the slides were stained for 15 minutes in 1 per cent. aqueous crystal-violet. They were then rinsed in tap water, average pH 7.5, and plunged into 95 per cent. ethyl alcohol for about 5 seconds, or half the length of time for which they were desiccated. From this they were transferred to an 80 per cent. alcohol, 1 per cent. iodine, and 1 per cent. potassium iodide solution for about 45 seconds, then passed slowly through 95 per cent. and absolute alcohol into a mixture of absolute alcohol and clove oil, equal parts. Differentiation in this mixture was observed under the microscope, and when correct the slides were passed through four changes of xylol and mounted in canada balsam. Observations were made with Zeiss 1.5 mm., 1.3 N.A. and 3.0 mm., 1.4 N.A. objectives, using a pointolite lamp. The preparations, already differentiated to the point where only the chromonemata are stained, very soon fade; they can, however, readily be restained.

OBSERVATIONS.

Somatic chromosomes.

The chromosomes of the first pollen-grain haploid division are shown in Text-fig. 1. Each chromosome is readily distinguishable by its size and the relative position of the 'attachment'. They can similarly be distinguished in all meiotic phases following diplotene. The letter designations A-E have been assigned to the five chromosomes as indicated.



TEXT-FIG. 1. Haploid chromosome complement at metaphase of first pollen-grain division, $\times 4,400$ and reduced to 3,000 in reproduction.

TEXT-FIG. 2 *a*. Late diplotene; *2 b* early diakinesis; *2 c* mid diakinesis; *2 d* late diakinesis; *2 e* metaphase. $\times 2,200$ and reduced to 1,500 in reproduction.

When brazilin stain is used and the staining includes the matrix of the chromosomes, as in Text-fig. 1, the 'attachment constriction' appears as a lighter staining or colourless constricted region. Differential staining

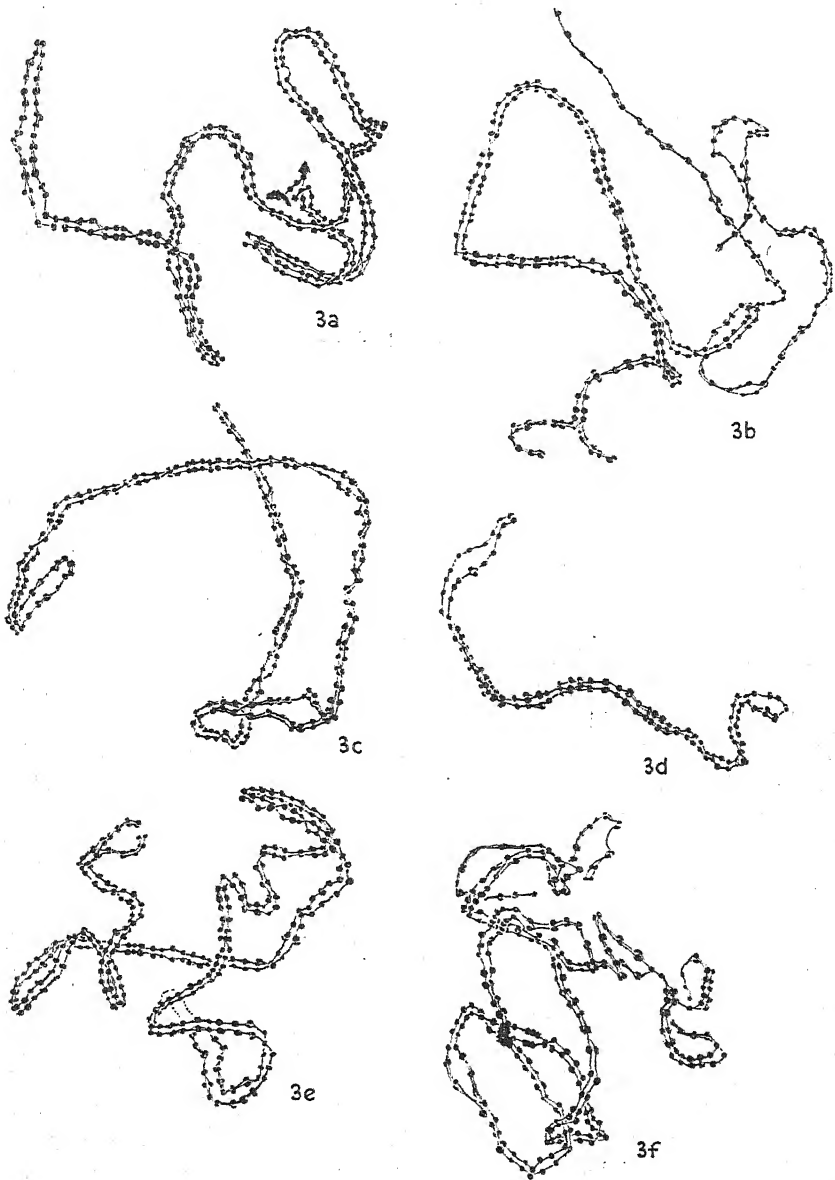
with crystal-violet shows clearly that the somatic metaphase chromosomes are longitudinally quadripartite, and the anaphase chromosomes longitudinally double in the first pollen-grain division, as maintained by Sharp (46), Hedayetullah (17), and others in the case of root-tip somatic chromosomes. Some further details of pollen-grain somatic chromosome structure are being published elsewhere in a study of the effects of X-radiation (23), and other studies are in progress.

Meiotic chromosomes.

Leptotene. At leptotene the fine chromosome threads or 'chromonemata' (using this term in its wider sense as 'the filamentous constituent of a chromosome' (47) without necessarily referring to its spirality) are distributed, apparently without any special orientation, throughout the nucleus (Pl. I, Fig. 1). At this stage the chromosomes consist of strings of 'ultimate chromomeres' (7, 8). The chromomeres vary considerably in size and in distance apart along the chromonema. They are circular in outline when viewed from any angle; no trace of split chromomeres (or paired chromioles) was observed prior to the onset of synapsis. In this respect *Trillium* is strikingly different from *Fritillaria Meleagris* (24).

Zygotene. Synapsis of (optically) unsplit chromomeres may apparently begin at any point along the length of the chromonemata, though there are indications that it most frequently begins near the attachment and at the ends. In general, only similar-sized chromomeres synapse, but unequal mates are occasionally observed. In conformity with the latter is the occasional observation of two unequal-sized synapsed pairs of chromioles in late pachytene. The general similarity in size of the chromomeres which synapse, and especially their similarity in homologous regions just prior to synapsis, indicates very strongly that they are not fortuitous thickenings of the chromonema but that they represent differently differentiated regions.

Seen in end-view the synapsed zygotene chromomeres are dumb-bell shaped. Before synapsis is completed in all regions, the earliest synapsed chromomeres begin visibly to split. There is therefore no definite zygotene stage for the whole nucleus. Fig. 2 of Pl. I illustrates mid-zygotene of our terminology. It is equivalent to amphitene of Janssens (25), there being both synapsed and single chromonemata. Figs. 5*a* and *b*, Pl. I, show parts of synapsed chromonemata. The attachment region is not visibly differentiated at this stage with the crystal-violet stain. Twists about each other occur at frequent intervals along the synapsed chromonemata. The impression is gained that this may be due to the chromonemata approaching one another from different levels, rather than to any torsion effect. The paired chromonemata cannot be traced throughout their entire length, and it is therefore impossible to make any definite statement on



TEXT-FIG. 3 *a-f*. Pachytene chromosomes of the nucleus illustrated in Plate I, Fig. 3, here drawn separately and more diagrammatically. Figs. 3 *a*, 3 *c*, and 3 *f* are complete bivalent chromosomes. $\times 4,400$ and reduced to 3,000 in reproduction.

the frequency of twists or overlaps, but they appear to be somewhat more numerous than chiasmata at diplotene or early diaphase. Successive overlaps from one plane possibly cancel each other in some cases before the

splitting of the chromomeres and the formation of chiasmata. No evidence of 'synizesis' was observed.

Pachytene. Pachytene likewise is not a definite stage for the whole nucleus. Pl. I, Fig. 3, illustrates mid-pachytene; most of the chromomeres are synapsed, but many are not yet split into chromioles, while others are already split and opened out in pairs. In Text-fig. 3 *a-f* lengths of the chromonemata are drawn separately. It has not been possible to pick out all five complete pairs, but three complete bivalent chromosomes are illustrated in Text-figs. 3 *a*, 3 *c*, and 3 *f*. It will be noted that in Text-fig. 3 *b* the lower end of the pair shows the diplotene separation, while the middle region is typical pachytene and the upper ends are not synapsed. This upper end is not, however, still in the leptotene or early zygotene stage, as some of its chromomeres have split. The two chromonemata are in widely separated optical planes in this region, and have apparently never synapsed. This splitting of chromomeres in single chromosomes which have failed to synapse is similar to observations on asynaptic oats and wheat (21) and *Fritillaria Meleagris* (24). In the latter case, however, there is evidence that the splitting is precocious and prevents synapsis, whereas in this case it occurs after the normal period for synapsis has passed.

At no period of zygotene or pachytene have we been able to see the *transverse* connexions between chromomeres found by Belling (9) in his preparations stained with brazilin. After the chromomeres have become tightly synapsed in mid-pachytene each pair divides to form four chromioles. In end view they form a rectangle, the distance between homologous pairs of chromioles being considerably greater than that between sister chromioles. In other words, the 'primary split' is much more distinct than the 'secondary split'. This condition continues into diplotene, where it is, of course, greatly enhanced.

When the chromomeres split, many adjacent pairs of chromioles are seen to be connected longitudinally by only a single thread, and this connexion may be either direct or tangential as shown by Belling (cf. Pl. I, Fig. 6). Similarly, when at a slightly later stage there is a thread connecting each chromiole longitudinally with its neighbour, all four threads may be parallel, or two of them may form a cross (cf. Pl. I, Fig. 9). We cannot say whether the pairs of threads are formed by the splitting of the original thread, or whether the one thread remains unsplit and a new one grows out to unite the chromioles lacking a connexion, as Belling maintains. The occasional observation of short threads which do not extend fully across the intervening space, favours Belling's view. The significance of such observations at the limits of microscopic resolution may, however, be questioned.

Belling (9, p. 396) states that he has identified both 'direct' and 'oblique' chiasmata in about equal numbers at pachytene in *Lilium*. We

are unable to satisfy ourselves that we can distinguish these with any degree of accuracy. Neither can we determine an increase in the length of the chromonemata between leptotene and pachytene as described by Belling (7, 8). It appears to us to shorten slightly in *Trillium*. In *Lilium*, two species of *Aloe*, and *Agapanthus*, Belling (6) found the number of ultimate bivalent chromomeres at pachytene to range from about 1,500 to 2,500. In *Aloe purpurascens* he found 1,248, but considered this probably an underestimate. In our preparations of *Trillium* the minimum is about 900, and the maximum about 1,000.

Diplotene. The diplotene stage starts with the beginning of the opening-out between pairs of paired chromatids. It appears that opening-out begins as soon as the paired chromomeres have split into tetrads of chromioles and there are four distinct longitudinal threads connecting them. The opening-out appears always to be between homologous pairs of chromioles, not between sisters. As the opening-out proceeds the crossed threads of late pachytene evidently develop into the clearly defined chiasmata of diplotene and later stages.

Pl. I, Fig. 4, illustrates very early diplotene. There are regions in which the pairs of paired chromatids are widely separated, and others in which opening-out has scarcely begun. At one point in this cell the chromosomes are massed together, apparently around the major nucleolus. This massed condition occurs only rarely in *Trillium*; it gives an appearance superficially like the leptotene 'bouquet stage' found in some animals. Opening-out often begins at the ends of the chromosomes; and it is evidently the chiasmata formed during pachytene which prevent it from proceeding along the entire length.

At the beginning of diplotene the chromioles are distinct and are approaching the size of leptotene or pachytene chromomeres. As contraction proceeds during diplotene the longitudinal connecting threads shorten and the paired chromioles lose their identity. By late diplotene or early diakinesis the contraction, possibly together with the accumulation of extra chromatin around the chromonemata, has obscured both the longitudinal chromomere differentiation and the quadripartite chromatid structure so that only a 'bivalent' structure remains (Text-figs. 2*a* and *b*). This 'bivalent' condition persists until mid-late diakinesis, at which time spiralling begins, as shown, for example, in Pl. II, Figs. 10 and 13.

While there is neither a definite 'second contraction' nor a definite 'diffuse stage' in this material, there is during diplotene both a general contraction and a measure of diffusion of chromosome outlines which renders analysis of chromosome behaviour through this period very difficult. The so-called strepsitene stage is not present in this or any other material we have examined. Superficially a twisted appearance results, of

course, as adjacent diplotene loops or internodes tend to lie in planes at right angles to each other.

Diakinesis. Early diakinesis according to our terminology is illustrated in Text-fig. 2*b*; mid diakinesis in Text-fig. 2*c*; late diakinesis in Text-fig. 2*d*; and metaphase in Text-fig. 2*e*. Differential staining shows the chromonema structure in the later diakinesis and metaphase chromosomes. Pl. I, Fig. 8 *a-d*, illustrates four bivalents, B-E respectively, from a single cell at mid-late diakinesis. Chromosome pair A was perpendicular to the focal plane and could not, therefore, be represented accurately. Between mid and late diakinesis a zigzagging of the chromonemata appears (Pl. I, Fig. 9, and Pl. II, Fig. 10), and by late diakinesis the paired chromosomes are seen to be composed of four very loosely spiralled or zigzagged chromatids or chromonemata. These spirals run in pairs, excepting where they change partners at the chiasmata. When the chiasmata are numerous the paired condition is somewhat obscured, and a very entangled appearance results (Pl. II, Fig. 11). By very late diakinesis or pro-metaphase each bivalent is very clearly seen (Pl. II, Figs. 11 and 12) to consist of two pairs of chromonemata which are spiralled in parallel excepting at the chiasmata, where they change partners, and in the region of the attachment. Near the attachment the chromatids lie parallel, but they are not spiralled. Photomicrograph 25*c* of Pl. III shows irregularities in the outline of the chromonemata in the large spiral which might possibly be taken to suggest a minor spiral structure within them. The four swollen regions which are seen in each half-gyre are, however, merely points of greater optical density, two of them being at the edges where the segment is perpendicular to the focal plane and two at positions where the chromatid spirals cross each other. The coiled chromonema does, however, twist about on its own axis, as suggested by Kuwada (28). (See especially his diagrams, Fig. 114 *a* and *b*.) This can most clearly be seen when the 'tertiary' split appears (Pl. II, Fig. 11).

The attachment regions appear as loops of paired chromonemata extending out on either side from the margin of the spirals of the tetrad or bivalent (see especially Pl. I, Fig. 8, and Pl. II, Fig. 11, and Photomicrographs 22 and 24 of Pl. III, in which they are marked with arrows). The chromonemata lie very close together in the loop, and often appear to form a single strand. Tangential views such as that of Pl. I, Fig. 8, reveal the doubleness, however, and it is particularly clear at metaphase in the A-chromosome bivalent shown in Pl. II, Fig. 14. The possibility of there being a single undivided body, invisible in these preparations, to which the 'spindle fibre' is 'attached' cannot, of course, be ruled out, but the 'attachment region' is clearly double, and on one side of the bivalent shown in Text-fig. 8*d* there are *two* bodies similar to the 'attachment chromomeres' of some authors. A repulsion between the 'attachments' of a tetrad is

apparent in the later stages of diakinesis, but in *Trillium* it is evidently too weak to cause any degree of 'terminalization'. It is noteworthy that chiasmata very frequently occur immediately adjacent to and on both sides of the attachment.

Pl. I, Fig. 9, is of a tetrad with four chiasmata; analysis of the relationship of the chromonemata in successive internodes of this tetrad shows clearly that they could not have originated from alternate opening-out along the reductional and equational planes as maintained by supporters of the classical theory, unless one assumes that there has been an additional chiasma which has broken—an assumption which is highly improbable for this material. Pl. II, Fig. 10, shows a tetrad with a single chiasma. Pl. II, Fig. 13, shows interlocked bivalents. If opening-out is reductional at the attachment, as most genetic and cytological evidence indicates, then, unless crossing-over has preceded this stage, the two paired chromatids of chromosome A which are interlocked in chromosome E must be non-sister chromatids, which, it is generally agreed, is impossible. On the other hand, if they are sister chromatids, this would, on the classical theory, involve the opening-out being equational not only at the attachment, but also in the loop adjacent to it, which is itself contrary to the theory. Were it not possible to follow the individual chromatids, this configuration would appear to be readily explicable on the classical theory, since the interlocked loop of chromosome A is the second one from the attachment. The significance of the configuration as evidence against the classical view, and in favour of partial chiasmotypy, would therefore be missed by the proponents of either theory.

In very late diakinesis and metaphase a further split becomes apparent in each of the four chromatids of a tetrad, Pl. II, Figs. 11 and 12. It is termed the 'tertiary' split, since the plane of union between homologues is commonly known as the 'primary split', and the pachytene division of chromomeres into pairs of sister chromioles as the 'secondary split'.

Pro-metaphase and metaphase. The chromosomes are similar in internal structure during these stages, and differ only in being aligned at the equatorial plate in the latter. For present purposes these stages are considered together. The outlines of the matrices which surround the spiral chromonemata may be seen in the photomicrographs, especially Pl. III, Figs. 22-4. By ordinary methods of preparation these would be the outlines of the 'chromosomes'.

The pairs of chromatids continue as parallel spirals, in these differentially stained preparations, excepting where they change partners at chiasmata. Changes of direction in coiling occur associated with chiasmata. Owing to the exchanges of partner, which prevent the formation of a closely coiled spiral, the direction of coiling is much more difficult to trace in detail at this stage than in anaphase, after the chiasmata have terminalized

or cancelled out, so that most studies of changes of direction have been made at the latter stage. Changes of direction at metaphase or early anaphase are usually associated with chiasmata, excepting in the cases of bivalents such as those in Pl. II, Figs. 14 and 15. Bivalents of these types require special consideration. In both Figs. 14 and 15 it is bivalent A, with almost terminal attachments which is illustrated. There are no chiasmata between the chromosomes, but they are held together at their ends in Fig. 14 and at the middle in Fig. 15. Fig. 14 is the type of bivalent considered by Darlington (13) to be held together by terminalized chiasmata, but in *Trillium* there is very little movement of chiasmata prior to anaphase, and at the one end it is the attachments that are still so close together. Now, at diplotene in *Trillium* it appears very evident that chiasmata are alone responsible for preventing complete separation of homologues, and it has been noted above that changes of direction in coiling are generally associated with chiasmata. It therefore seems significant that there are two changes of direction, marked X, in the coiling of each of the four chromonemata of the bivalent in Pl. II, Fig. 14. That of Pl. II, Fig. 15, has one change each in two of its chromatids and two each in the other two. These and other similar configurations we can best interpret as having had pairs of compensating chiasmata (the complexities in this interpretation of Fig. 15 will be referred to later) which prevented separation of the homologues at early diplotene, but which with the subsequent contraction of the chromosomes cancelled each other, leaving the terminal association dependent upon either a specific attraction or upon a cohesive effect of the matrix. A terminal attraction, independent of chiasmata, is postulated by Belling (7-9). Darlington (13) considers that all interstitial chiasmata terminalize without cancellation. Metz (36, 37) stresses the idea of a 'chromosome sheath' holding chromosomes together. Schultz (3) considers that it is the matrix which holds the chromosomes together after terminalization of chiasmata. Pl. II, Fig. 19, illustrates a late metaphase bivalent of chromosome B with three chiasmata involving changes of direction of coiling, and, apart from these, a change of direction, near the middle, in two sister chromatids. The significance of this configuration for theories of coiling will be discussed later.

Anaphase. As the attachments of the homologous chromosomes repulse each other or are attracted towards the poles the chiasmata are resolved and the four chromonemata become widely separated. The longitudinal tertiary split in each chromonema becomes especially evident at this time. The attachment, or at least its central region, loses its staining capacity to some extent, as shown particularly clearly in Pl. II, Figs. 16 and 17, of chromosomes C and E respectively. The tertiary split was not obvious in the chromosomes illustrated in Figs. 16, 17, and 18 at the time these figures were first drawn, excepting in parts of chromosomes

A and B in Fig. 18. Upon restaining after the preparation had faded, the split was clear in all of them, but they have not been redrawn to show it.

During anaphase the spirals assume their most compact form and the changes in direction of coiling can then most satisfactorily be studied, Near the attachment there is still often difficulty in determining them; elsewhere they are obvious, as shown particularly in Photomicrograph 27, Pl. III.

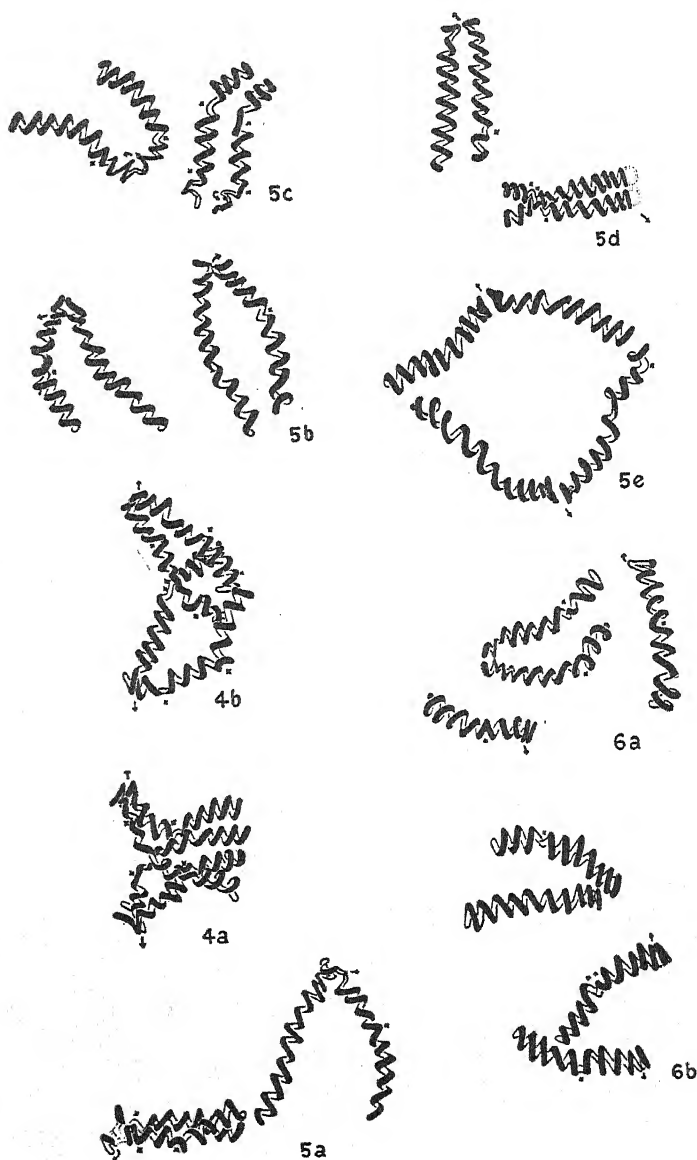
In Pl. II, Fig. 18, all five pairs of first anaphase chromosomes are drawn separately, and it may be noted that changes in direction of coiling are usually in equivalent positions on two of the four chromonemata. In Text-figs. 4-6 various anaphase configurations of chromosome A are illustrated with special reference to changes in the direction of coiling. They will be considered in detail later.

The number of changes in the direction of coiling, as counted at anaphase, appears to be about twice the number of chiasmata at metaphase as determined from preparations which were not destined to show chromonemata. There are thirty-two changes in the anaphase shown in Fig. 18. A slight, possibly significant, decrease in the number of chiasmata from early diakinesis to metaphase was found in studies of fully stained preparations (Table I). This may be due in part to two chiasmata being counted as one, as must happen in any studies in which the whole chromosome including matrix is stained, but it is probably due in part also to reciprocal compensating chiasmata cancelling each other, or to one member of a pair of non-compensating chiasmata being cancelled out, in which case, if the cancellation occurs after coiling has begun, changes in direction of coiling may apparently remain to mark the position formerly occupied by the chiasmata. (See especially Pl. II, Figs. 14 and 15.)

TABLE I.
Chiasmata per Nucleus.

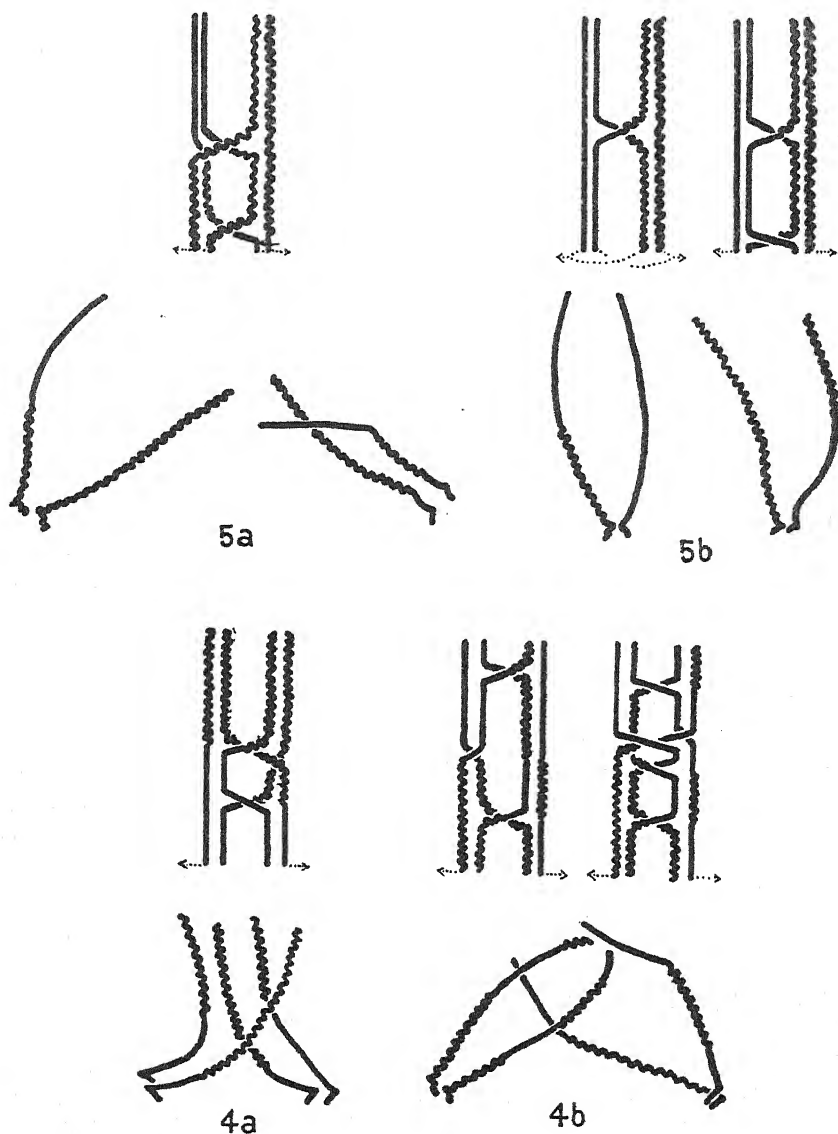
Stage.	Number of nuclei.	Total number of chiasmata.	Mean.	Standard error.	Number of terminal chiasmata.
Mid diaphase	10	165	16.5	± 0.608	20
Late diaphase	20	343	17.15	± 0.465	47
Metaphase	20	288	14.4	± 0.447	38

In these preparations of *Trillium* there is no trace of a minor or 'primary' spiral running along the length of the chromatids at right angles to the major spiral, as shown by various Japanese workers in *Tradescantia*. (See especially Kuwada and Nakamura (30, Text-fig. 3) for diagrammatic illustration.) Since each chromatid of *Trillium* is longitudinally divided by the 'tertiary' split before the anaphase, the minor spiral, if it exists at all in *Trillium*, must be sought in each half-chromatid. In two abnormal



TEXT-FIGS. 4-6. Anaphase configurations of chromosome A illustrating changes in direction of coiling. Figs. 4*a* and *b* early anaphase. Figs. 5*a-e* anaphase. 6*a* and *b* unpaired chromosomes at early anaphase. $\times 4,400$ reduced to $3,800 \times$ in reproduction.

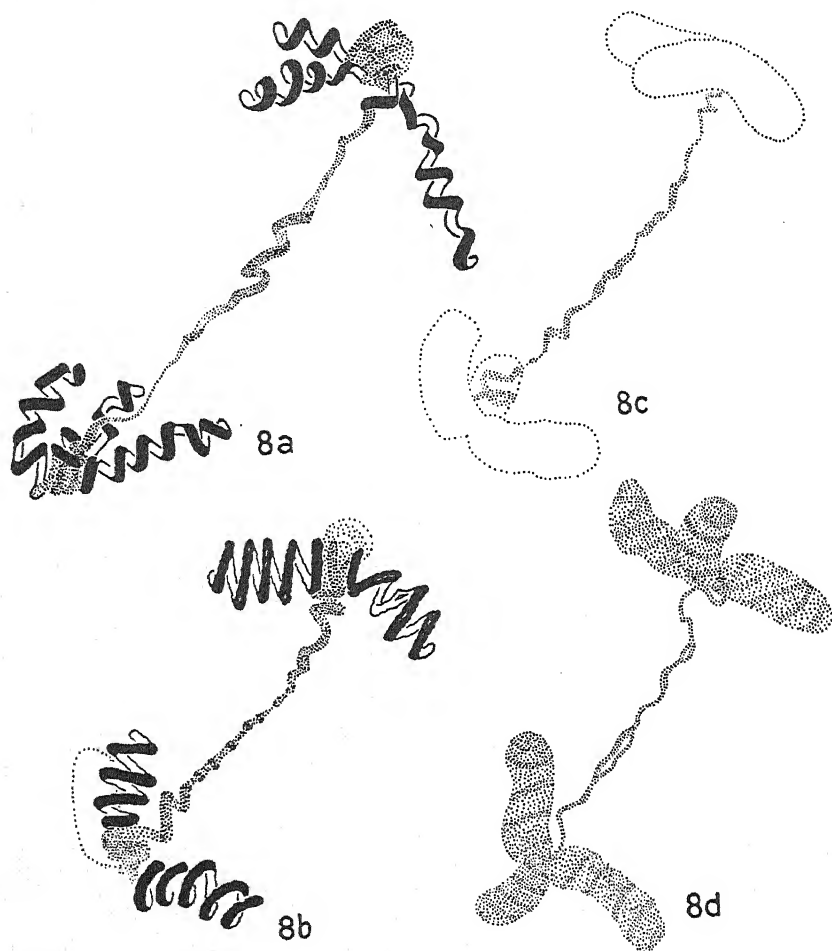
plants which form 'bridge' chromatids through crossing-over in an inverted segment (see Smith, 50) a small-gyred spiral, somewhat like the minor spiral reported in *Tradescantia* is regularly seen (see Text-figs. 8*a-d*). The spiral in Text-fig. 8*c* in particular is very like that of Kuwada and



TEXT-FIG. 7. Diagrams illustrating possible interpretations of configurations 4a and b and 5a and b. — = dextrorse coiling; ~ = sinistrorse coiling. Left-hand diagrams represent the configurations seen at anaphase. Right-hand diagrams represent pro-metaphase configurations which could, on the hypothesis being tested, have given rise to them. Alternative interpretations, essentially similar, are possible in most cases. As shown, configuration 5b could be taken to indicate equational separation at the attachment, though most genetic and cytological evidence is against this possibility.

Nakamura's Pl. VIII, Fig. 9, and Shinke's (48) Pl XVII, Fig. 7. In the *Trillium* preparations it is, however, quite clear that the small-gyred spiral of the 'bridge' chromatids has resulted from the drawing-out of the major

spiral. The length of the chromatid segment involved is known ordinarily to constitute about six major spiral gyres. In Text-figs. 8 *a* and *b* which are anaphase figures, and Text-figs. 8 *c* and *d* which are telophases, various



TEXT-FIG. 8 *a-d*. 'Bridge' chromatid arising through crossing-over in a heterozygous inverted segment in chromosome D—a condition found in two corms. Figs. 8 *a* and *b* anaphase. Figs. 8 *c* and *d* telophase. Showing various stages in the drawing-out of the major spiral. In Fig. 8 *d* the tertiary split also is particularly clear. Magnification 4,400 reduced to 4000 in reproduction.

stages in the drawing-out of the major spiral gyres are illustrated. The 'tertiary' split was particularly clear in the preparation illustrated in Text-fig. 8 *d*. In Text-fig. 8 *b* the two ends of the 'bridge' chromatid are clearly coiled in opposite directions, as would be expected from its mode of origin whenever the paired homologues from which it arose were coiled in opposite directions. It is therefore possible that there may have been some cancel-

lation of coiling to give the straight region seen at the middle of this figure. Whether or not opposite spirals usually cancel on stretching must depend upon the consistency or elasticity of the chromonema, concerning which very little is known.

Second or homotypic metaphase. There is practically no interkinesis in *Trillium*. The first anaphase chromosomes pass without material change of structure to the second metaphase. It is, however, more difficult to get clear figures of the chromonemata at the homotypic metaphase as the matrix stains more deeply than in the preceding anaphase. Photomicrograph 29, Pl. III, is the best second metaphase obtained; it may be compared with the first anaphase of Photomicrographs 27 and 28. There is no indication of a further split occurring in the chromonemata during the second meiotic division.

Second anaphase and telophase. During the second anaphase and telophase each spiral chromonema appears single (Photomicrograph 30, Pl. III). The tertiary split of late diakinesis, so clearly seen at first anaphase, which will function as the plane of separation in the first pollen-grain division, i.e. in the second nuclear cycle after its initiation, is difficult to see in the homotypic metaphase or anaphase, but it becomes very clear again in the telophase. The second telophase chromosomes in one nucleus of a pollen tetrad or quartet are shown in Pl. I, Fig. 7.

Unpaired chromosomes. Two unpaired homologous chromosomes were found occasionally at early anaphase in a number of different plants. For the present study their chief significance is for the interpretation of changes of direction in coiling and for the determination of the time of chromosome splitting. Those shown in Text-figs. 6*a* and 6*b* were lagging near the equator after the remainder of the chromosomes had gone to the poles. They have various changes of direction, and each of them was clearly quadripartite in the early anaphase, though no attempt has been made to illustrate the tertiary split in these black and white figures, since they were drawn only to show changes in the direction of coiling.

Chromatid fragments. Chromatid fragments evidently arising through crossing-over between a normal and an inverted chromosome segment were found in two plants, mentioned above, which apparently belonged to the same clone. The tertiary split is clearly seen in these fragment chromatids through both meiotic divisions, but no separation of the chromatid halves occurs during meiosis. They are described in detail in a separate publication (50).

DISCUSSION.

Spiral structure. Analysis of this unusually favourable material seems to clear up several contradictions and apparent contradictions in the interpretation of chromosome structure by various workers. In the first place,

it is quite certain that we are here dealing with a true spiral chromonema in all stages of meiosis following diakinesis. The alveolar interpretation of many earlier workers cannot be admitted. This has, of course, already been made evident by a considerable number of recent workers who find the early interpretation of spiral structure by Baranetzky (2), Bonnevie (10, 11), and others to be substantially correct. Though it is somewhat invidious to select a few from the many recent authors who have demonstrated the spiral structure of chromosomes, the works of Kaufmann (26), Sakamura (43), Kuwada (28), Maeda (33), Sharp (46), Shinke (48), Sax (44), Tuan (55), Taylor (53), and Nebel (39), may be cited as examples.

There is considerable confusion in the discussions of the details of spiral structure through variability of terminology, and particularly from loose usage of the word 'chromosome'; further confusion has arisen from the tendency of several authors to assume that conditions observed in mitosis must necessarily apply in meiosis, and vice versa. We refer to paired chromosomes at stages from late pachytene to anaphase either as chromosome pairs, bivalents, or tetrads. A tetrad is composed of two chromosomes or dyads; each chromosome or dyad of two chromatids. The 'tertiary' split divides each chromatid. These halves will be referred to as half-chromatids, not, for the present at least, by any new, distinctive term since it seems probable that the chromatids are the 'effective' linear cytogenetic units. A double spiral composed of two chromatids coiled in parallel is termed a 'chromosome spiral', and the single spirals are 'chromatid spirals'. Fujii (15), according to Shinke (49), Kuwada and Nakamura (30, 31), and Shinke (49) consider, as mentioned above, that each chromatid spiral is itself composed of a very fine, thread-like spiral which has its gyres at right angles to those of the larger spiral; each chromatid spiral is a 'double-coiled single spiral'—K. and N. (30). The small-gyred spiral has been termed the secondary spiral by some authors and the primary by others, including Fujii (15). We term the large spiral the major spiral, and in discussing the question of whether or not a 'primary spiral' exists in this material we shall refer to it as a 'minor spiral'.

Kuwada (28) has shown that for chromatid spirals to separate without entanglement it is essential that the chromonema should twist on its own axis once in each gyre of the major spiral. In some papers it is difficult to determine whether the authors are referring to this twist, or to the 'primary spiral' of Fujii. In *Trillium* this 'Kuwada twist' is readily seen, but the minor spiral is not present in the form illustrated by Kuwada and Nakamura (30) in their Text-fig. 3. Nebel (38), however, maintains that each heterotypic anaphase chromatid is longitudinally split in *Tradescantia*, as we find in *Trillium*, and he further considers (39 and unpublished) that each *half-chromatid* constitutes a minor spiral. He interprets

in this way the photographs of Kuwada (29) and of Kuwada and Nakamura (30), though these authors seem to imply that each *chromatid* is a single minor spiral. The half-chromatids of *Trillium* are twisted and often wavy in outline, but they do not appear to be real spirals. Their form when seen singly is such as would be expected from their being twisted about on their own axis when they are together in the major spirals (see later discussion of the mechanism of spiralization).

According to Kuwada (28 *et seq.*), Fujii (15) was the first definitely to state that each chromatid forms an individual spiral. Previously it was more generally held that each dyad forms one large spiral, though Bonnevie (10) and Kaufmann (26) had shown that the spirals were double in some places. Single chromosome spirals are illustrated by Maeda (33) in a study of *Lathyrus*, Sax (44) in *Secale*, Taylor (53) in *Gasteria*, Nebel (39) in *Tradescantia*, and others, but in most cases the authors show double spirals, i.e. chromatid spirals in other illustrations, or state that they are found in parts of the chromosomes. It is in chromosomes like those of *Trillium*, in which resolution of chiasmata ordinarily does not occur before anaphase, that the chromatids are widely separated and show clearly their individual coiling. In chromosomes whose chiasmata terminalize before coiling is definitely established the sister chromatids apparently tend to coil together, thus giving the appearance of a single spiral. Kaufmann, in particular, notes the very close parallelism of the double spirals in *Tradescantia*.

One pair of changes in the direction of coiling of the chromonemata is figured by Taylor (53). Sax (44) noted that the (apparently single) spiral chromonemata of *Secale* coil in opposite directions in the paired dyads, and that sometimes they seem to reverse their direction of coiling near one end. Nebel (39) reports that in the *Tradescantieae* the spirals may turn in opposite directions on either side of the attachment. Darlington (13, p. 33) states that each chromatid is one spiral, with which we agree, but he continues: 'there is no reason to doubt that this spiral is single and turns in one direction.' He considers Sax's reversal of coiling to be an optical error due to his having taken one chromatid spiral as the continuation of the other. This criticism is surely unjustified. For one thing, it would imply that the sister chromatids are coiled throughout their length in opposite directions. Again, p. 290, Darlington states that 'The chromosome spiral seems to be a regular one. Were it to change direction in the middle of a chromosome arm an otherwise unaccountable irregularity would be expected in the outline of the chromosome such as is never observed at mitosis or meiosis.' In *Trillium*, changes in the direction of coiling are numerous, they apparently occur anywhere along the length of the chromatids and they cause no 'unaccountable irregularity' in the outline of the chromosome when it is stained in the ordinary manner. We find, as did Belling, that coiling begins in diakinesis, not at pachytene as

Darlington maintains. It is not completed until after the resolution of the chiasmata. In *Trillium* resolution is not ordinarily completed until anaphase. In the absence of chiasmata, i.e. in unpaired chromosomes such as those of Text-figs. 6 *a* and *b* or in tetrads lacking them, as in chromosome B, Pl. III, Photomicrograph 24, and chromosome A, Figs. 14 and 15, Pl. II, and also in free ends of tetrads with restricted chiasma formation, Pl. III, Photomicrographs 22, 23, and 25, there is a strong tendency for the coils of the two chromatid spirals to fit closely into one another and so to appear like a single spiral. It is doubtless this that has led to the single spiral interpretation of many authors.

The mechanism of spiralization.

The problem of the cause of spiralling and of the changes in the direction of coiling may next be considered. Darlington (13, pp. 289-92) argues that torsion causes both crossing-over and 'spiralization'. 'A regular spiral' (which he assumes) 'implies that its development is directed or controlled from one point. This is presumably the spindle attachment. . .'. A twisting of the thread is postulated as the simplest way in which control could be exerted. 'If the spiral arises in this way it should turn in the opposite direction on the two sides of the spindle attachment.' This possibility has been tested, he says, but the evidence adduced (that of irregular linear contraction in a ring chromosome) is clearly inadequate in view of (*a*) Taylor's (53) observation of changes in direction of coiling and of spirals, either similar or opposite, on the two sides of the attachment, (*b*) Nebel's (39) similar observations, and (*c*) the data here presented. It is evident that the changes in direction of coiling can occur anywhere along the length of the chromosomes, and that no single torsion mechanism located either at the attachment or elsewhere can account for spiralization; further, a torsion mechanism would lead to great difficulties in chromosome separation.

Koshy (27) in a study of somatic chromosomes of *Allium* states that the anaphase chromosomes are double and that their halves are twisted about each other in opposite directions on either side of the attachment. He dismisses, for what appears to be an inadequate reason, Kuwada's (28) suggestion that for each turn of the spiral there is a twist of the two threads about each other in the opposite direction, stating that 'A double spiral of this type cannot normally originate by the longitudinal fission of the spirally coiled parent chromonema'. This statement, in the first place, involves the assumption that the fission is initiated while the chromonema is coiled, whereas we find fission and coiling starting at about the same time. Even were that assumption justified, Nebel (40) has made it clear that Kuwada's suggestion is physically sound; it would require only division in any one plane. The mitotic anaphase chromosomes of *Allium* are not

large, and accurate determination of the direction of coiling in them must be very difficult. Studies of somatic chromosomes of *Trillium* (Huskins and Hunter (23) and unpublished) do not agree with Koshy's observations; the chromonemata in *Trillium* are sometimes twisted about one another, but not in the regular manner he describes.

Kuwada's (28) interpretation of the method of coiling appears to be the only one that will satisfactorily account for the easy separation of coiled double chromonemata. It is the structure which a double strand will assume if forced into a spiral with its ends more or less fixed. Observational evidence for it was found in unpaired ends of chromosomes, see especially Photomicrograph 25, Pl. III. In addition to this, limited though fairly definite evidence was found that the half-chromatids are similarly twisted about each other shortly after the tertiary split first appears, see Pl. II, Figs. 11, 12, and 18 *b*. This is, of course, a division cycle in advance of that considered by Kuwada,

As shown above, we find the tertiary split becoming visible at late diaphase, so that meiotic bivalents or tetrads are longitudinally eight-partite. Correlatively, in mitotic divisions the somatic chromosomes are very clearly four-partite before metaphase. In both mitosis and meiosis the spiralling begins more or less coincidently with the appearance of the first signs of the split in the chromatids. In both, the spiralling becomes tightest and most regular during the anaphase. It is perhaps a reasonable assumption, supported in part by observational evidence (though the value of this in fixed and stained material must be limited), that growth in thickness of the half-chromatids occurs during metaphase and anaphase on the outer surface of the chromatid. If such is the case and the half-chromatids are twisted about each other, then growth must be occurring spirally around them with a reversal of direction once in each gyre of the chromatid spiral, and will be on opposite sides of each half-chromatid in each successive gyre. The newly laid down extra-chromatin of each half-chromatid will sooner or later be expected to undergo syneresis, and this will provide a very simple self-perpetuating mechanism for spiralization as each half-chromatid, or in turn each chromatid, will be in a state of tension on alternate sides. In addition to the tension produced by syneresis there will be the heterogonic growth *per se*. The tension will be distributed spirally around each chromatid and will be on opposite sides as many times as there were gyres in the spiral in the previous division. A simple though too crude model, having tension on alternate sides, though not changing gradually from one to the other, can be made by placing the ends of two pieces of rubber tubing in a vice, then stretching one and binding the two together with wire for a short length, then stretching the other and continuing the binding and alternation of stretching. On being released this double tube will assume a zigzag form similar to that seen in

early diakinesis, and with very slight pressure, possibly analogous to pressure which could be exerted by a matrix or sheath on the chromonemata, will readily condense into a spiral of the type postulated by Kuwada. This simple hypothesis of the mechanism of spiralization seems surprisingly adequate in explaining Trillium data, as will be shown. It has also the general merit of demanding no special unknown torsion force, nor any genetically determined direction of coiling which might be liable to be disturbed by crossing-over, and it does not involve any strong tendency towards intertwining and entangling of chromatids as any torsion hypothesis must. On the other hand, it allows for random changes and a measure of inexactitude in the direction of coiling and for occasional entangling of chromatids such as have been observed in somatic chromosomes. The same arguments can be applied, *mutatis mutandis*, to somatic chromosome coiling, though the danger of adducing a general theory from observations limited to meiosis in a few species of plants is, of course, realized. The experimental evidence on the nature of the spiral structure in *Tradescantia* obtained by Kuwada and Nakamura (30, 31) through the action of ammonia is in complete agreement with our observations in *Trillium* regarding the major chromosome and chromatid spirals, but, as stated above, we find no evidence of a chromatid minor spiral in *Trillium*. It appears in fact as if the *chromatid* minor spiral of the Japanese authors and our 'tertiary split' may in some cases be alternative interpretations of the same optical images. It is the evidence provided by regions in which the tertiary split is too wide to be an optical illusion that determines our general interpretation. Kuwada and Nakamura (30) find only four chromonemata in a bivalent, but Shinke (49), although adopting their *chromatid* minor spiral interpretation, shows clear evidence of the tertiary split, i.e. of the eight-partite structure, in his Pl. XVIII, Figs. 37 *b*, 38, and 44. The *Tradescantia* *half-chromatid* minor spiral of Nebel ((39) and unpublished, personal communications) is not incompatible with our observations, but we interpret it as a twist and waviness rather than as a spiral in *Trillium*.

The cause of changes in direction of coiling. As mentioned, there appear to be about twice as many changes of direction of coiling at anaphase as there are chiasmata at diakinesis or metaphase. With very few exceptions, changes occur in similar positions on two of the four chromatids. These pairs of changes may be on chromatids going either to the same or opposite poles. Chromosomes that have been paired previous to the initiation of coiling usually coil in opposite directions along the paired parts of their length. The simplest unified hypothesis by which the changes of direction might be explained is that paired homologous chromosomes coil in opposite directions, and that in consequence two of the four chromatids must change their direction at each chiasma. This hypothesis has been found to fit some configurations very well, but it is evidently

inadequate as a general explanation. The diagrams presented in Text-fig. 7 illustrate the method of analysis. No attempt is made to present all the possible interpretations.

In Pl. II, Fig. 18, the five pairs of first anaphase chromosomes are drawn in the relative positions in which they occurred in the cell. Chromosome pair B at the top has, reading from left to right, 1, 3, 3, and 1 changes in direction of coiling in its four chromatids. In this order the chromatids will be referred to as 1, 2, 3, 4 in this figure. In chromosome pair A there are no changes in chromatid 1, and two each in chromatids 2, 3, and 4, if the double change in chromatid 3 is included. In chromosome pair C there is probably one change in each of chromatids 1 and 4, but owing to chromatids 1 and 2 being turned up it is very difficult to interpret them. Chromosome pair D probably has 0, 2, 3, and 1 changes of direction, but the stretching of the chromonemata near the attachment has rendered interpretation difficult in 1 and 2. Pair E has 1, 0, 2, 0, 3, 2, 1, and 1 changes of direction in its eight arms, but the identity of the chromatids on either side of the attachment cannot be determined with certainty.

The changes of direction of coiling in chromosome B of Pl. II, Fig. 18, can readily be interpreted as due to the occurrence of three chiasmata. Those in chromosome A can be similarly interpreted, but anomalies of direction of coiling in sister chromatids then result. If the double change in chromatid 3 is ignored and only two chiasmata postulated the anomalies are avoided. The uncertainties involved in the interpretation of chromosomes D, C, and E of Pl. II, Fig. 18, prevent interpretation of the origin of their changes of direction.

On Belling's hypothesis of crossing-over it is the non-crossing chromatids at each chiasma which are the genetic cross-overs. Belling's hypothesis, of course, includes but goes beyond the general partial-chiasmatype theory of Janssens which has been analysed in detail and clarified by Darlington (13). On the partial chiasmatype hypothesis itself it is impossible to say which of the four chromatids at any given chiasma are the genetic cross-overs. They will be referred to in this section as crossing and non-crossing chromatids, without any genetic implications being made.

Text-figs. 4-6 show changes in direction of coiling in anaphase configurations of chromosome A taken at random from different nuclei. This, being the terminally attached chromosome, is the easiest one to interpret. Text-fig. 4 *a* is a very early anaphase tetrad in which there is one change in each chromatid. This configuration introduces difficulties in any precise interpretation of the significance of the changes in direction. To explain it on the assumption that chiasmata alone are the cause of changes it is necessary to assume two diagonal or non-compensating chiasmata, one of which has been resolved. The chromonemata which cross in the present configuration were initially involved in different members of the pair of

chiasmata assumed. This interpretation is shown in Diagram 4 *a*, Text-fig. 7. The direction of coiling being the same in all four chromatids at each end indicates, however, that though chiasmata may be directly responsible for the occurrence of changes of direction, the relationship is not a simple one, as it would be were homologous chromosomes always coiled in opposite directions. It is necessary to assume in this, as in several other instances, that similar direction of coiling has become established in both homologues in an unpaired, in this case distal, segment and that it has influenced the coiling of the chromatids in the paired segment, adjacent to it. The assumption of a third reciprocal chiasma adjacent to the attachment and therefore resulting in a twist rather than a distinct change of direction of coiling, would permit construction of a diagram in which coiling of homologues would be in opposite directions at the attachment end, but since the attachment region itself is not coiled this seems an unnecessary assumption. Text-fig. 4 *b* (and Photomicrograph 26, Pl. III) illustrates a very early anaphase in which there are eight changes of direction of coiling. This configuration, though complicated, has its changes in pairs situated in equivalent positions, and they can be interpreted as due to four chiasmata of which three have cancelled out. The four chromatids are, however, spiralled in the same direction along considerable parts of their length. The assumption of four chiasmata would possibly explain this if at the proximal chiasmal one crossing and one non-crossing strand had changed direction. The assumption of only two chiasmata would, however, explain this configuration better, see Text-fig. 7. Four of the changes would then be independent of or only indirectly related to chiasmata. Text-figs. 5 *a-e* are later anaphase configurations of chromosome A. The changes of direction in Text-fig. 5 *a* can be interpreted as due to two non-compensating chiasmata with one crossing and one non-crossing chromatid having changed direction at the proximal chiasma. Text-fig. 5 *b* requires for its interpretation on this hypothesis either the assumption of an additional chiasma near the attachment or equational separation at the attachment. Otherwise, sister chromatids must be assumed to have been coiled in opposite directions for at least half of their length, see Text-fig. 7. The changes in Text-figs. 5 *c* and 5 *d* can be interpreted without difficulty as due to three and two chiasmata respectively. The bivalent illustrated in Text-fig. 5 *e* has no clear changes of direction excepting one very near the distal end of one chromatid, but there are suggestions of a change in two chromatids near the attachment. To explain the wide separation of the sister chromatids in this configuration it seems necessary to assume the occurrence of a chiasma very close to the attachment (and therefore causing only a twist and not a definite change of direction) which has had to be drawn out or terminalized during anaphase. This assumption leaves unexplained the occurrence of the single change near the distal end. It is, however, so near to the free end that it

may easily have been distorted in separation ; it may therefore lack significance for the general problem.

The A-chromosome bivalent illustrated in Pl. II, Fig. 14, has two changes in each of its chromatids. These could be interpreted as due to two pairs of compensating chiasmata which have cancelled out, but all four chromatids coil in the same direction. In Pl. II, Fig. 15, there are 1, 1, 2, and 2 changes of direction on the four chromatids. These are not explicable as due to chiasmata unless there have been changes at the points indicated with a question mark, where there are irregular twists in chromatids 1 and 2. If these represent changes that have been nullified by a counter-direction of coiling set up in the proximal region the interpretation of this figure would be practically the same as that of Pl. II, Fig. 14.

As mentioned above, coiling is much more difficult to study at metaphase than at anaphase, but a metaphase bivalent of chromosome B is illustrated in Pl. II, Fig. 19. The short arms are turned up and are therefore too difficult to interpret accurately. In the long arm there are 1, 3, 2, and 0 changes of direction in the four chromatids. Two pairs of these are clearly associated with chiasmata, but the exact relationships are difficult to determine near the attachment. The changes near the middle of the arm in two associated chromatids are not associated with a chiasma, nor does it seem possible that they could have been caused by a chiasma present earlier. At the left end of this bivalent the homologues are coiled in opposite directions. Between the attachment and the central pair of changes they are coiled in the same direction. This is evidently the cause of the changes in this case.

The changes of direction are fairly certainly not due solely to chiasmata. It appears probable that many of them do originate from the occurrence of chiasmata between homologues coiled in opposite directions, but that some changes, such as the *single* one in Text-fig. 5 e, may have a fortuitous origin, and on the other hand, that homologues sometimes coil in the same direction and chiasmata between them do not then always produce changes of direction. These two minor variables, one positive and one negative, may numerically cancel each other, and so account for the number of changes being approximately equal to twice the number of chiasmata. It appears fairly evident that a chiasma may sometimes cause one crossing and one non-crossing chromatid to change direction, in other words, either member of a pair of sister chromatids may determine the direction of coiling of both. If paired homologues are coiled in the same direction at one end and in opposite directions at the other, a pair of changes will obviously result independently of chiasma formation.

The mechanism envisaged for the origin of coiling would account for the general tendency of homologues to coil in opposite directions, but would allow also for exceptions. The zigzag produced by tension on

alternate sides would have no strong tendency for coiling in any one direction, but contact between the homologues may cause their coiling to be in opposite directions. Unpaired lengths of chromosomes could freely coil in the same direction. Further work on this problem of coiling will be undertaken later.

Text-figs. 6*a* and 6*b* illustrate unpaired chromosomes with changes of direction. There is one clear change of direction in each chromatid of Text-fig. 6*a*, and a possible second change near the attachment in one of them. If these chromosomes have never been paired, the change is a fortuitous one at similar positions in the two chromatids of each chromosome. If they have been paired, they must have had two complementary chiasmata situated very close together. Text-fig. 6*b* has 1, 0, 2, and 1 changes in its four chromatids. These chromosomes have probably been paired; otherwise sister chromatids were coiled in opposite directions along one-third to one-half of their length at the distal ends, which is improbable. It seems that these chromosomes may have been associated by a pair of reciprocal chiasmata with one crossing and one non-crossing strand changing at either one or both chiasmata.

If these chromosomes have been paired it is difficult to see why cancellation of chiasmata should have permitted their falling apart in these cases, whereas in other cases bivalents without chiasmata remain united, as in Pl. II, Figs. 14 and 15, of chromosome pair A, and Photomicrograph 24, Pl. III, of chromosome B. The changes in direction of coiling in these latter paired chromosomes seem to indicate that they have had two reciprocal chiasmata which have cancelled each other. In any event, either a special terminal affinity or a cohesive force of the matrix must apparently be involved in their remaining paired at metaphase. It is possible that the closeness together of the assumed chiasmata in Text-figs. 6*a* and 6*b*, as determined by the position of the changes of direction, may have caused early cancellation.

The mechanism of crossing-over.

Practically all the observations of prophase stages here recorded are in substantial agreement with those of Belling (9 and earlier); in detail we differ in our inability to detect *transverse* connexions between chromomeres at pachytene or to determine the frequency of 'direct' and 'oblique' chiasmata at that stage. Our evidence indicates that crossing-over occurs at the time of visible splitting of the chromosomes, that is, at late pachytene, as maintained by Belling, but it does not necessarily support his hypothesis in detail. The crossing-over hypothesis of Sax (44, 45) and the 'classical' conception of alternate opening-out along the reductional and equational planes, on which it is based, are both incompatible with the present observations. Interlocked chromosomes, Pl. II, Fig. 13, and the

tracing of individual chromatids, such as those in Pl. I, Fig. 9, disprove alternate opening. The absence of terminalization before metaphase, the unravelling of chiasmata without breaking at anaphase, and the cancellation of compensating chiasmata at anaphase or earlier eliminate the mechanism invoked by Sax for crossing-over. Correlatively, Hearne and Huskins (16) have shown prophase terminalization without diminution of chiasma frequency in *Melanoplus*. On the other hand, in studies of the effects of X-rays, Huskins and Hunter (23) have found that when a single chromatid is fragmented its ends will unite with the two chromatids of other chromosomes lying near it. It therefore seems possible that breaks and rejoins may occasionally occur in untreated material in the manner postulated by Sax, but probably only before the matrix is established. They would most probably result in unequal crossing-over.

Chromosome structure and the mechanism of mitosis and meiosis.

Bonnevie (10) and other early workers showed that somatic chromosomes are quadripartite at metaphase and double at anaphase and telophase. Their observations were discounted by many cytologists as optical illusions due to vacuolization (see 56, pp. 138-9). More recently Sharp (46), Hedayetullah (17), and others have shown double structure at anaphase in root-tips of plants with large chromosomes. Although Darlington (12) described a quadripartite chromosome structure in the pollen-grain mitosis of the Scilleae, he has more recently (13) discounted all such observations as optical illusions due to the 'hollow nature of the chromosome'. He now considers that splitting occurs during the 'resting stage'. In the pollen-grains of *Trillium* (see 23) the quadripartite metaphase structure is extremely clear. In *Anthoxanthum* the quadripartite nature is clear in end views of root-tip metaphase chromosomes (19, Fig. 15 *a, b, c*). Darlington's three arguments against splitting occurring in the division preceding separation (13, p. 47) may all readily be countered. Merriman (35) and Nebel (40) believe that the somatic telophase chromosome is quadripartite, and Taylor (52) considered the meiotic telophase chromosomes to be eight-partite. We have not been able to find this extra split in *Trillium* pollen-grain or meiotic chromosomes.

From observations on a number of normal and aberrant types of plants and animals, including especially *Trillium*, *Fritillaria*, *Avena*, *Matthiola*, and *Melanoplus femur-rubrum*, a working hypothesis which seeks a common principle of chromosome behaviour in mitosis and meiosis was formulated by Huskins (20). This 'mitosis-meiosis' hypothesis arose as a modification of Darlington's 'precocity theory' but is not acceptable to him. It differs from the precocity theory in its primary observational basis of the time at which chromosomes split, and it has very different implications when applied to special cases. Applied to Beadle's (4 and

earlier) polyploid maize, for instance, it does not (as Beadle might be taken to imply) lead to the assumption that pairing must occur in the supernumerary divisions, while the precocity theory does. It will account for univalent chromosomes dividing twice during meiosis in cases where there is a large number of them and they delay the division. The tertiary split will account for the repulsion between chromatids at the heterotypic telophase. Either hypothesis will account for most features of the long-chromosome *Matthiola*—cf. Armstrong and Huskins (1). The mitosis-meiosis hypothesis seems more readily capable of extension to include cases of metaphase association without chiasmata such as occur in the Diptera. Its basic assumption is that chromonemata, including the attachment region, are attracted to one another in pairs *at all stages* of both mitosis and meiosis (instead of only at prophase as the precocity theory postulates), and that pairs of pairs repulse each other at all stages.

Darlington (14) has recently found that the autosomes of the male *Drosophila pseudo-obscura* are associated at metaphase without chiasmata, and Stevens (51), Metz (36), Huettner (18), and others have made similar observations in *D. melanogaster* and other Diptera. Darlington now calls this 'anomalous chromosome pairing'. It seems more probable that it is a normal type of pairing for some at least of the chromosomes in a fairly large number of organisms. The problem to be considered is whether it can be explained on the same principles as pairing in chromosomes having chiasmata. To explain it Darlington assumes three repulsion forces and two forces of attraction. With such a set of variables the theory can neither be proved nor disproved, and his precocity theory, in our opinion, thereby loses one of its chief merits as a working hypothesis.

In view of the diversity of the forms of meiosis and mitosis and of chromosome pairing it is *a priori* very doubtful if any simple hypothesis can have universal application. It seems, however, that the simple hypothesis of an attraction in pairs and a repulsion between pairs of pairs may be worth further testing, and observations are therefore being continued on a number of diverse organisms. For the present it may be pointed out that if different chromosomes in the same or different organisms may differ to the extent of a whole division cycle in their time of splitting, the hypothesis will cover many apparently anomalous cases. For instance, the very close pairing found by Painter (42) in salivary gland cells of old *Drosophila* larvae would be due to the inhibition of chromosome splitting (in the sense mentioned later herein) in these cells which have, he considers, undergone their final mitosis. The same might apply to some of the staminal hair cells of *Tradescantia* and other hair cells studied by Belar (5) and Belling (9) (which the latter emphasized 'would not normally divide again') and so render understandable the otherwise almost inexplicable difference of opinion between these two outstanding cytologists on the one hand, and

on the other those who have found somatic chromosomes of root-tips or pollen grains to be double in the anaphase. The occurrence of splitting in parts of the chromosomes would on this hypothesis prevent these parts pairing in meiosis. *Fritillaria Meleagris* (24) gives evidence for this. The 'm' chromosomes of *Alydus* (56), the small chromosomes of *Yucca flaccida* (41), and the autosomes of male *Drosophila* and other Diptera would remain paired without chiasmata, because the secondary split which normally occurs in pachytene would not have occurred until prometaphase. The preponderance of compensating over non-compensating chiasmata in *Melanoplus* (16) would be due to splitting occurring at a slightly later, more condensed stage than in the Liliaceae.

In animal tumours there are often striking differences from the normal in the wideness of the split in chromosomes at a given stage of mitosis. There is variation in the time of chromosome splitting in relation to the formation and functioning of the spindle mechanism, cf. especially Ludford (32). On the mitosis-meiosis hypothesis relative delay in splitting would cause synapsis and crossing-over in somatic cells, and from this loss of whole chromosomes, or, in structural hybrids, homozygous sectional duplications and deficiencies might result. If these involved genes necessary for the normal progress of mitosis, abnormal tissues would arise. Premature splitting would also cause chromosome loss.

Pairing between non-homologous chromosomes in the absence of normal homologues, found by McClintock (34) in maize, supports the general mitosis-meiosis or precocity conception very strongly. The normal pairing of homologous chromosomes and the secondary association of bivalents in polyploids indicates, of course, that besides the more general attraction force postulated in these hypotheses there is a specific attraction between homologous units. Only further examination of diverse types of meiosis can show whether Darlington's precocity theory or this modified hypothesis has any general validity, but the simple concept of attraction in pairs and repulsion between pairs of paired chromosome threads has already proved a useful working hypothesis in the analysis of several problems. The mitosis-meiosis hypothesis carried to its logical conclusion must, however, assume meiosis to be initiated by failure of the customary split of the chromatids in the last premeiotic division (20), and this premeiotic separation of unsplit chromosomes involves a departure from the basic principle of the hypothesis. If the hypothesis is to stand at all, the doubleness or singleness of the threads must be a physiological and reversible state; observations of single unsplit chromonemata, as at leptotene, will then have to be taken as indications of the physiological condition, not as evidence that the thread has never been split. This would not be surprising in view of (a) the apparent doubleness of mid-diaphase bivalents which earlier are clearly seen to be quadripartite, or (b) the

apparent singleness of the homotypic anaphase chromonemata which were seen to be divided by the tertiary split during the heterotypic division and become clearly double again at the homotypic telophase, but it obviously limits the value of the concept.

SUMMARY.

T. erectum L. has five pairs of very large morphologically distinguishable chromosomes.

In pollen mother-cell meiosis chromomere structure is very clear through the leptotene, zygotene, and pachytene stages, which may occur coincidentally in different pairs of the same chromosome. The total number of chromomeres is between 900 and 1,000; they vary considerably in size. No trace of doubleness or splitting of chromomeres was observed prior to the onset of zygotene.

Chromomeres split soon after pairing, and then fall in pairs of paired chromioles. The longitudinal connexions between them may be either direct, or tangential. Transverse connexions could not be detected. Twists or overlaps at zygotene appear to be more numerous than chiasmata at diplotene. In early diplotene the homologues appear to be held together only by chiasmata.

With differential destaining the four chromatids of each bivalent can be traced individually throughout their length at diakinesis and metaphase. Configurations are found in both single and interlocked bivalents which are incompatible with the 'classical' concept of alternate opening-out.

Spiralling of chromatids begins in diakinesis. In the attachment region sister chromatids extend out as *double* loops on either side of the bivalents. Elsewhere they become spiralled in parallel excepting for the exchanges at chiasmata. Paired chromosomes usually coil in opposite directions; in unpaired ends all four chromatids may coil in the same direction. Changes of direction are usually associated with chiasmata, and are therefore related, though probably only facultatively, to crossing-over.

At anaphase, changes of direction of coiling occur anywhere along the length of the chromosomes, and almost invariably in equivalent positions on two of the four chromatids. Any spiralization hypothesis based on a unitary torsion force seems to be ruled out by the changes of direction. A working hypothesis based on the concept of heterogonic growth in a double spiral is presented.

Unpaired chromosomes sometimes have changes of direction of coiling so situated as to indicate that they have had compensating chiasmata which have cancelled each other. On the other hand, occasional bivalents are found remaining paired at metaphase without chiasmata. The matrix therefore appears to be significant in the maintenance of metaphase pairing.

There is very little movement of chiasmata prior to anaphase. During the anaphase chiasmata are resolved by either terminalization or cancellation.

A 'tertiary' split, which will function as the plane of separation in the first pollen-grain division, becomes visible before the heterotypic metaphase.

No splitting of the chromosomes is observed in the homotypic division. The homotypic anaphase chromosomes, which have been longitudinally divided by the 'tertiary' split of the heterotypic prophase, form single spirals, but their doubleness becomes obvious again at telophase.

In the first pollen-grain division the somatic chromosomes, including the attachment region, are longitudinally quadripartite before metaphase, and double in the anaphase.

Chromatid fragments and chromatids with two attachments were found in two plants; they apparently arise through crossing-over in a heterozygous inverted segment. When the chromatids with two attachments are stretched across the plate, the major spiral is stretched out into a small-gyred spiral similar to that which some authors have interpreted as a 'primary' spiral running at right angles along the major spiral.

Since each chromatid in *Trillium* is longitudinally divided by the tertiary split, the primary spiral interpretation in its present formulation for *Tradescantia* by Kuwada and Nakamura is not valid for *Trillium*. Each half-chromatid is, however, irregularly waved or twisted, and may possibly with some treatments be found to constitute a minor spiral, as Nebel maintains. It does not exist in any regular spiral form in the present material.

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EXPLANATION OF PLATES I-III.

Illustrating Professor C. Leonard Huskins's and Mr. Stanley G. Smith's paper on 'Meiotic Chromosome Structure in *Trillium erectum* L.'

PLATE I.

All magnifications originally 4,400, but reduced in 3,400 in reproduction.

- Fig. 1. Leptotene stage in a pollen mother-cell of *Trillium erectum* L.
- Fig. 2. Zygotene.
- Fig. 3. Pachytene.
- Fig. 4. Beginning of diplotene.
- Fig. 5 a and b. Segments of individual chromosome pairs at late zygotene.
- Fig. 6. Segment of a chromosome pair at early pachytene.
- Fig. 7. Pollen tetrad at telophase; chromosomes shown in one nucleus.
- Fig. 8 a-d. Chromosome pairs B, C, D, and E respectively, at mid-late diakinesis. Preparation destined to show only the chromonemata.
- Fig. 9. Chromosome pair B illustrating the beginning of spiralization at mid-late diakinesis.

PLATE II.

Magnification 4,400.

- Fig. 10. Chromosome pair C, illustrating an early stage of spiralization at mid-late diakinesis.
- Figs. 11 and 12. Chromosome pairs E and B respectively, showing spiral form and the tertiary split at very late diakinesis or prometaphase.
- Fig. 13. Chromosome pairs A and E interlocked at mid diakinesis.

Figs. 14 and 15. Chromosome pair A at metaphase, illustrating double nature of attachment region, changes in direction of coiling, and association of homologues at metaphase without chiasmata.

Figs. 16 and 17. Chromosomes C and E at anaphase; illustrating particularly the loss of staining capacity at the 'attachment', and the 'tertiary' split near the attachment in fig. 17.

Fig. 18 *a-e*. Complete set of anaphase chromosomes drawn separately for analysis of changes in direction of coiling. The tertiary split has been illustrated in chromosomes B and A at the right.

Fig. 19. Chromosome pair B at late metaphase illustrating chiasmata and changes of direction of coiling.

PLATE III.

Photomicrographs: magnification *ca* 1,800 excepting Fig. 20 which is *ca* 1,000 and Fig. 25 *c* which is *ca* 3,600.

Fig. 20. Pachytene.

Fig. 21. Very late diakinesis or prometaphase.

Figs. 22-4. Metaphases. Figs. 22 and 24 show the looped form of the attachment region, and fig. 24 shows a bivalent of chromosome B remaining paired without chiasmata.

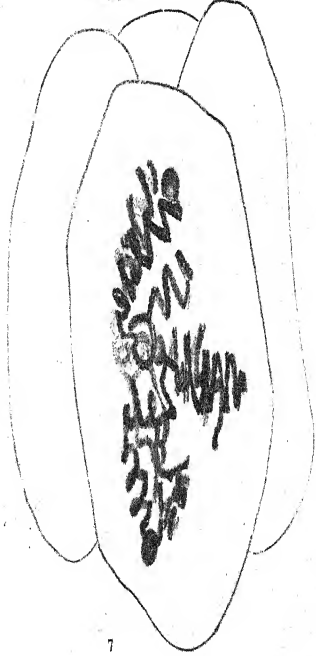
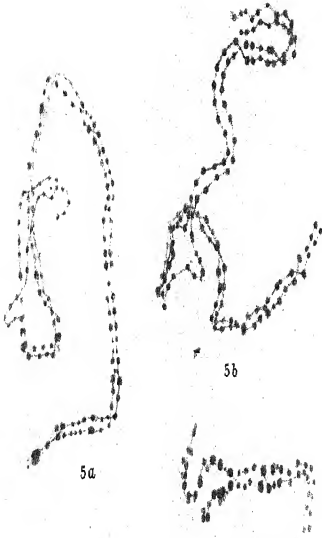
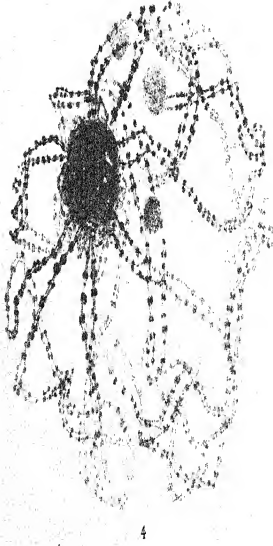
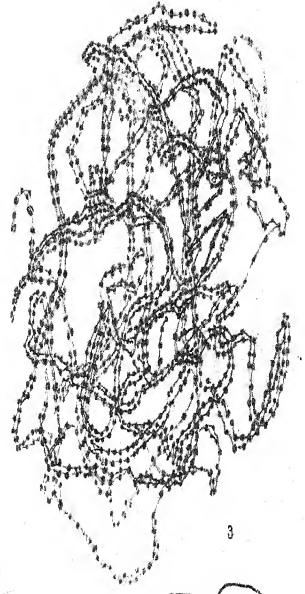
Fig. 25 *a-c*. Illustrating spiral structure at prometaphase. Figs. 25 *a* and *b* are taken at different focal levels, and fig. 25 *c* is an enlarged composite print from the two negatives.

Fig. 26. Early heterotypic anaphase.

Figs. 27 and 28. Heterotypic anaphases showing changes in direction of coiling.

Fig. 29. Homotypic metaphase.

Fig. 30. Homotypic anaphase.





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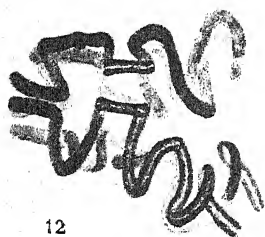
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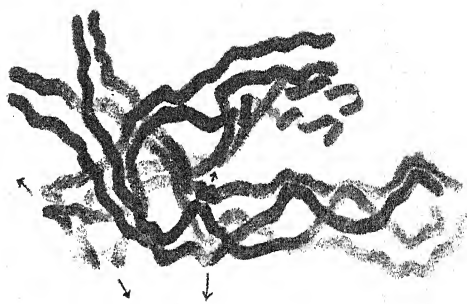
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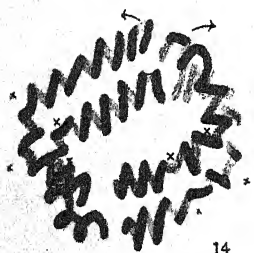
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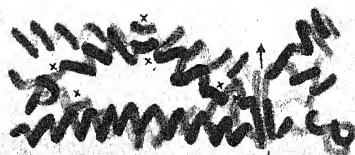
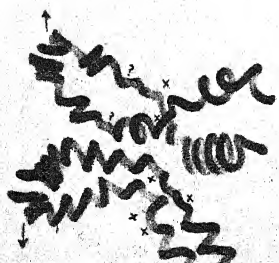
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13



14





"B"



"A"



"C"



"D"



"E"



b



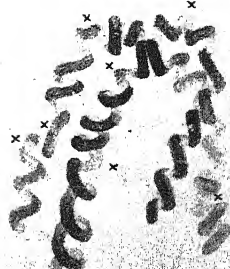
a



c



d



e



20



21



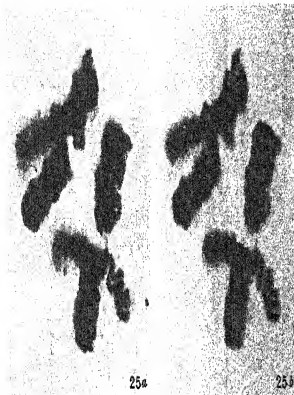
22



23



24



25a



25b



25c



26



27



28



29

Cytological Aspects of Physiological Sterility in *Coprinus sterquilinus* Fr.¹

BY

J. E. SASS.

(Department of Botany, Iowa State College, Ames, Iowa, U.S.A.)

With Plate IV.

THE failure of normal sporulation on otherwise well-developed fruit bodies has been observed in a number of Hymenomycetes. This phenomenon may be considered a form of sterility, in the sense that very few viable spores are produced. The known cases seem to fall into two classes, genetic and physiological. A fixed type of sterility was found by the writer (5) to occur in a *Coprinus*, probably *C. boudieri* or a closely allied species. In this plant sterility is associated with the failure of the nuclear divisions which normally occur in the basidium after the fusion of the dikaryon. Such sterility is genetic, and is quite independent of nutritional or other environmental conditions.

Buller (3) reviewed the results of studies conducted in his laboratory on sterility in *C. lagopus*. It was found that partial, variable sterility occurs in monosporous (haploid) mycelia of this heterothallic species. In the same species, physiological sterility also was observed in properly mated diploid cultures, when too many fruit bodies developed simultaneously in the culture. The latter phenomenon has been studied by the present writer in *C. sterquilinus* Fr. This fungus is grown conveniently in pint bottle cultures on sterilized horse dung. Two or three weeks after the medium is inoculated, many fruit body rudiments can be discerned. In the volume of culture medium used in this study, one to five fruit body initials begin to enlarge rapidly, forming a caespitose group. If all but one or two of these fruit bodies are removed the remainder enlarge and sporulate in a normal manner. If several fruit bodies are permitted to enlarge they exhibit varying degrees of sterility. Old cultures that have produced several crops are almost certain to yield highly sterile fruit bodies.

¹ This investigation was aided by a grant from the Rockefeller Fluid Research Fund, for which grateful acknowledgement is made.

GROSS FEATURES OF STERILE FRUIT BODIES OF *C. STERQUILINUS*.

Young fruit bodies are turgid in both fresh and exhausted cultures, and no way has been found to distinguish in advance those fruit bodies that are to become sterile. Fruit bodies in fresh cultures remain turgid until autodigestion occurs, whereas those in old cultures become visibly flaccid relatively early, and become markedly wilted during the period when normally, rapid expansion and a turgid condition would be observed. Spore development is initiated on all fruit bodies, and for some time it is impossible to predict whether a given hymenium is to become fertile or sterile. On the pure white areas of large sterile fruit bodies the spores may attain nearly full size, but remain colourless. On the brownish areas which occur on the gills of sterile fruit bodies the spores become brownish black. In spore smears made from the dark patches on the gills of sterile fruit bodies, most of the spores exhibit various shades of pale brown and fail to germinate. The few black spores present in the smear germinate. Clamp-bearing mycelia and fertile fruit bodies are produced from the viable spores under favourable nutritional conditions.

OBSERVATIONS ON NUCLEAR CONDITIONS.

A cytological study of first-crop fruit bodies was made to determine the normal nuclear cycle. Fruit bodies from old, exhausted cultures were studied in order to gain an understanding of the nuclear phenomena associated with physiological sterility. In all of the numerous young fruit bodies which were examined, the basidia were found to be at first binucleate, and subsequently to become uninucleate by fusion of the dikaryon (Pl. IV, Figs. 1, 2, 3). Most of the older fruit bodies subsequently exhibit the nuclear history typical of the Hymenomycetes. Two nuclear divisions occur in the basidium, which remains four-nucleate for a considerable period preceding the appearance of sterigmata (Pl. IV, Figs. 4, 5, 6). Four spores develop on each basidium. The four nuclei remain on the basidium, near the apex, until the spores have attained nearly full size (Pl. IV, Figs. 7, 8). Subsequently, one nucleus migrates into each spore. This nucleus divides at least once in the spore (Pl. IV, Fig. 9).

Occasional fruit bodies, especially those collected from old cultures, yield preparations in which *four sterigmata are present on binucleate basidia*. Very few basidia contain four nuclei. The nuclei of these rare tetrads are of about the same size as the nuclei of normal tetrads (Pl. IV, Fig. 6). The nuclei of the binucleate basidia are considerably larger than the tetrad nuclei (Pl. IV, Figs. 10, 11, 12). Occasional basidia contain one large and two small nuclei (Pl. IV, Fig. 12). Regardless of the nuclear condition, four sterigmata and four spores arise on each basidium of a sterile fruit body (Pl. IV, Fig. 12). Most of these spores do not attain full size and do not receive nuclei.

DISCUSSION.

Sterility in the Hymenomycetes can be divided for the present into two categories, genetic and physiological. Genetic sterility in haploid fruit bodies produced on unmated mycelia of heterothallic species, is homologous with sterility in haploid angiosperms. Genetic sterility in diploid species may be attributed to genetic inhibition of meiosis in the basidium. In the category of physiological sterility, the available evidence indicates that inadequate nutritional conditions may merely prevent the full development of hymenial elements, or may produce marked aberrations in the nuclear mechanism of the basidium.

An interesting aspect of the latter situation is the formation of four sterigmata and spores of considerable size on basidia in which only one division of the fusion nucleus had occurred. The generally accepted account of the formation of sterigmata states that the four centrosomes, after functioning during the second division, migrate to the apex of the basidium and determine the position of the sterigmata. In the writer's preparations of *C. sterquilinus* centrosomes are clearly evident from the later prophases of the fusion nucleus through the first division. Subsequently the centrosomes disappear. In the basidia of sterile fruit bodies, the second nuclear division does not occur. Nevertheless four sterigmata develop on each basidium. It should be recalled that two sterigmata can arise on a basidium containing four nuclei (Bauch, 1; Sass, 4), and even on multinucleate basidia (Bauch, 2). These facts call for a re-examination of the role of the centrosomes in the nuclear mechanism of sporulation.

SUMMARY.

1. Sterile fruit bodies arise on diploid, homothallic, clamp-bearing mycelia of *C. sterquilinus* Fr. when the culture is overcrowded with several simultaneously expanding fruit bodies, or after a culture has produced several crops.

2. Sterile fruit bodies are flaccid; they may be entirely white, or may have brown to blackish areas on the gills. The white gills bear undeveloped spores of variable size. The dark areas bear spores of full size, varying in colour from pale brown to black.

3. A few of the black spores are viable, producing clamp-bearing mycelia on which fertile fruit bodies are formed.

4. In the basidia of sterile fruit bodies, usually only one division of the fusion nucleus occurs. The two diad nuclei can be distinguished from the rare tetrad nuclei by the much larger size of the former.

5. Four sterigmata and spores arise on basidia which contain two nuclei. Nuclear migration does not occur from the basidium to the spores.

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5. ———: The Cytology of a Diploid, Sterile Hymenomycete. Mycologia, xxiv. 229-32, 1932.

EXPLANATION OF PLATE IV.

Illustrating Dr. Sass's paper on 'Cytological Aspects of Physiological Sterility in *Coprinus sterquilinus* Fr.'

Photomicrographs taken with a Leitz 70 × (2.6 mm.) fluorite objective and Periplan oculars. Magnification of illustrations approximately 400.

Fig. 1. Basidia containing fusion nuclei in zygotene, showing bivalent strands, some of which exhibit spiral twist.

Figs. 2, 3. Early prophase of fusion nucleus; chromatin in diffuse condition. Note *nebenkern*, in various positions with respect to nucleus, and containing several crescent-shaped 'Golgi bodies'.

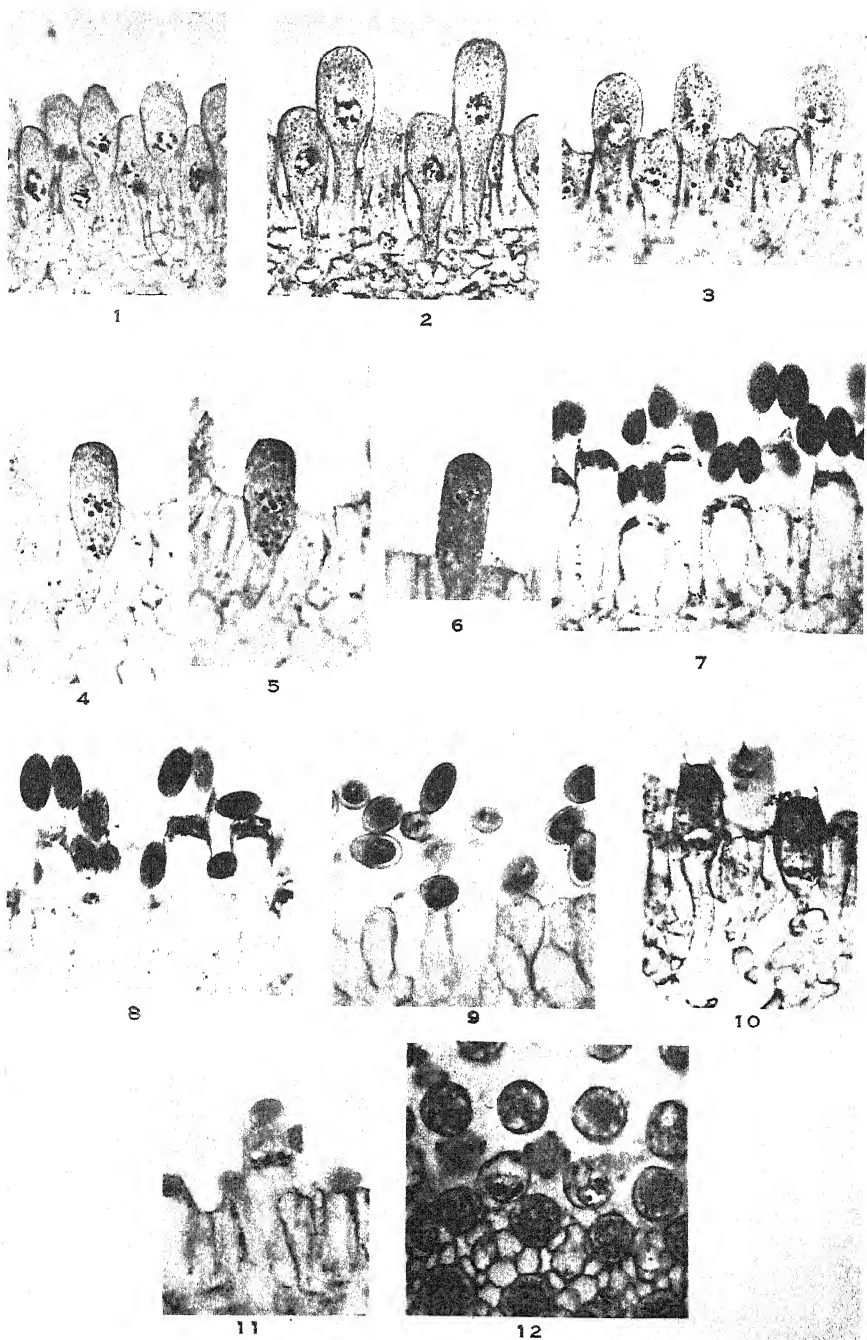
Figs. 4, 5. Basidium photographed at two foci, showing four nuclei resulting from two divisions of fusion nucleus. Remnants of *nebenkern* at base of basidium.

Fig. 6. Tetrad of nuclei in basidium.

Figs. 7, 8. Normal sporulation. Each basidium contains four nuclei, not all of them visible because of inadequate depth of focus.

Fig. 9. Binucleate spores, nearly mature.

Figs. 10, 11, 12. Binucleate, 4-sterigmatic basidia of sterile fruit body.



SASS - STERILITY IN COPRINUS.

Huth, Stubbs X. Kent.

The Fructification, *Calathiops* Bernhardt, n. sp.

BY

M. BENSON.

With Plate V and three Figures in the Text.

THIS fine specimen of a seed-bearing pinna of a Pteridosperm is the first that has been found to illustrate adequately how the seeds were borne in *Calathiops* fructifications.

Single seeds were found by Jongmans (7) growing in the cupules of *Calymmatotheca*, and other cases have been recorded by Gothan and Zimmermann, but the early stages remained unknown. Prof. Seward was unable to determine from his Kentucky specimen that the fertile frond of *Diplotmema furcatum*, recently described, was a *Calymmatotheca* or a *Calathiops* (9).

Recently the author was able to demonstrate that the ovular fructification of *Sphenopteridium bifidum* could be linked by a series of specimens from a *Calathiops* to a *Calymmatotheca* stage (2), but the bulk of *Calathiops* specimens show little external trace of seeds and, up to the present, have been generally interpreted as pollen synangia. The form-genus *Calathiops*, founded in 1864 by Goeppert (5), owing to its publication in a treatise dealing with the Permian Flora was allowed to lapse, and the term has only recently been revived by Gothan (6). He suggested that *Calathiops* (Goeppert) was comparable with *Calymmatotheca* (Stur) and *Telangium* (Kidston) ((1) and (8)). Gothan has now most generously allowed me to describe this new specimen which proves his surmise to be correct and that *Calathiops* is an ovular structure. The specific name is that suggested by Gothan in honour of the Collector, K. Bernhardt. The specimen was originally placed in the genus *Pterispermostrobus* (Stopes (10)), but Gothan changed the name to *Calathiops* on recognizing that the first name was merely a duplicate.

Owing to our ignorance up to the present time of the nature of the *Calathiops* fructification and the long time that has elapsed since it was diagnosed, some revision of Goeppert's diagnosis seems called for.

With Gothan's approval I propose the following as the revised diagnosis of *Calathiops*, Goeppert.

Naked, fertile pinna presumed to belong to a Pteridosperm. The pinna bears pedicillate, cupulate ovules which, when immature, are crowded together on the more or less sympodially produced ultimate arms. The



TEXT-FIG. 1. A drawing from the stone of the whole specimen of *Calathiops Bernhardti*, n. sp. A-A, Branching of the main rachis; B-B, Transverse ridges on the rachis; C-C, Insertion of immature ovules; D-D, Elongated valvate lobes of the cupules. The dotted lines indicate the edges of flaked surface and the striated line the margin of the slab.

ovules, when mature, escape from the cupules, while the pedicels are still short, as in *C. Bernhardti*, or, occasionally, after their elongation, which results in a Calymmatotheca fructification. Thus a Pteridosperm ovular apparatus may be, when immature, a Calathiops and, when mature, a Calymmatotheca. In the great majority of species so far described, amounting now to five and twenty, the fructification is immature.

Calathiops is an interesting type of fructification, as it has been shown to contain very immature ovules, e.g. in *C. Schuetzia* (Benson (3)), but at the same time to exhibit a high degree of cuticularization of the cupular

lobes. The macerated-out, bladder-like epidermal cells of these cupules gave rise to the view that these mummified ovular tufts were pollen synangia (Benson (4)).

Description of Calathiops Bernhardtii, n. sp.

Locality. The following record is given by Gothan: 'Vorhalle near Hagen (Westphalia) Ziegeli (Verblendstein—Werke)'.

Geological horizon. Gothan states:—
'Upper Namurian, below the so-called "Mager" coal layers of the Ruhr basin.'

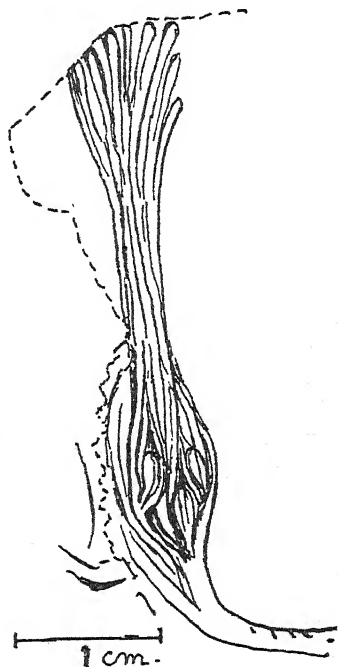
Attribution. The accompanying flora in the horizon, though marine, is very rich and in part beautifully preserved. It cannot be established with certainty to which plant genus the specimen belongs. Owing, however, to the very distinct transverse ridges shown on its rachids and on those of *Mariopteris acuta* it belongs more probably to that than to any other plant.

The fine black marine clay slab on which the pinna is inscribed measures roughly 23×16 cm. A photograph of the whole specimen taken while it was on loan at the British Museum is given in Pl. V, Fig. 1, and drawings from the stone are added to exhibit some of the details of the branching more clearly (Text-figs. 1 and 2).

The mature seeds are not preserved, but the moulds of those which were being liberated measure from 2.75 to 3 mm. in length and a little under 2 mm. in width.

In various places young ovules can be seen at the base of their cupules and are obviously attached. The cupules reach a length of 32 mm. and their lobes are valvate. The rachis is provided with small transverse ridges well known in certain Sphenopterids, as *Mariopteris*, *Sphenopteris striata*, and allied forms, though especially clearly in *Mariopteris* species.

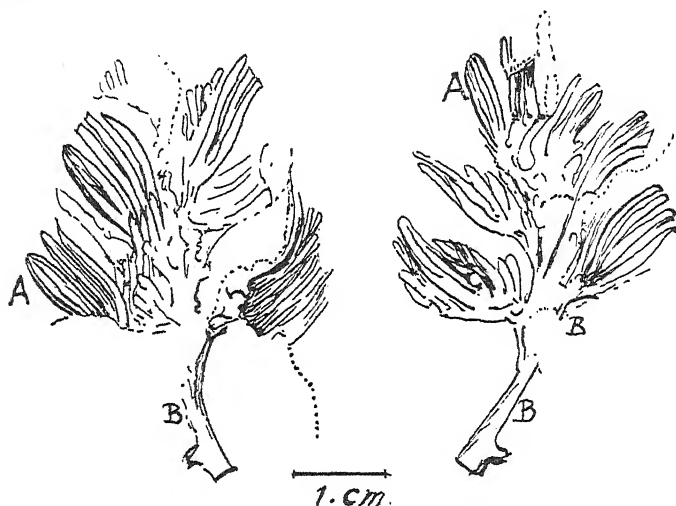
The rachis shows evidence of repeated forking in different planes, and was therefore of a quite distinct character from the bifacial fronds of recent ferns. The ultimate ovular groups show a considerable resemblance to such fructifications as *Whittleseya elegans*, the structure of which it is hoped to discuss in a later paper.



TEXT-FIG. 2. An enlarged drawing from the stone at c' in Text-fig. 1, to show the sympodial character of the terminal tufts and the ovules on the inner sides.

Calathiops Gothani, n. sp.

I append to the above description of *C. Bernhardtii* the description of a new species of the genus, which I have great pleasure in naming after Prof. Gothan. A photograph of the two counterparts is given in Pl. V, Fig. 4.



TEXT-FIG. 3. (*Calathiops Gothani, n. sp.*). Drawings from the two counterparts of the specimen showing the younger phase of a species somewhat similar to *C. Bernhardtii*. Owing to the forkings of the rachis occurring in different planes the cleavage has left an uneven surface which renders the figures inadequate without reference to the original. At A-A the cupular lobes are still closed together; B-B are parts of the forking rachis.

The specimen was found in 1933 and differs in geological horizon from *C. Bernhardtii*, as it occurred in the Teilia Quarry, Flintshire, N. Wales—a well-known black limestone locality of the Calciferous Sandstone series and thus is Lower Carboniferous—*C. Bernhardtii* is much more recent.

The pinna is at a younger phase, and indications of attached ovules within the cupules are not very distinct. I cannot identify it with any previously described specimen, but it resembles rather closely in the insertion and spreading character of the cupular lobes and cupules the species *C. Bernhardtii*.

One of the counterparts has been deposited in the Geological Dept., British Museum (Nat. Hist.), and has the registered number V. 23775.

Diagnosis of Calathiops Gothani, n. sp.

A Lower Carboniferous species of *Calathiops*, of which the only specimen so far recorded consists of two counterparts procured from the black Limestone (Calciferous Sandstone Series) of the Teilia Quarry,

Flintsh., N. Wales, in 1933. The terminal series of cupulate ovules on the forking rachis closely resemble those of *C. Bernhardtii*, but the cupules measure only 10–11 mm. in length instead of 32 mm.

SUMMARY.

This paper records two new species of *Calathiops* (Goeppert). They are at two different stages and from two different geological horizons. Their importance lies in the fact that for the first time this genus reveals to ocular inspection its true nature as a seed-bearer. On the strength of this and much other evidence recently acquired and shortly to be published, a revised diagnosis of Goeppert's genus has been drawn up.

Diagnosis of *Calathiops* (Goeppert). Naked, fertile pinna presumed to belong to a Pteridosperm. The pinna bears pedicillate, cupulate ovules which, when immature, are crowded together on the more or less sympodially produced ultimate arms. When mature (i.e. ready for pollination) the ovules escape from the cupules, while the pedicels are still short, as in *C. Bernhardtii*, or, occasionally, after their elongation, which results in a *Calymmatotheca*-like fructification.

My warmest thanks are tendered to Professor Gothan for lending his valuable specimen for the prosecution of the research on the reproductive bodies of the Pteridosperms which I have in hand, and for assistance in its description.

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EXPLANATION OF PLATE V.

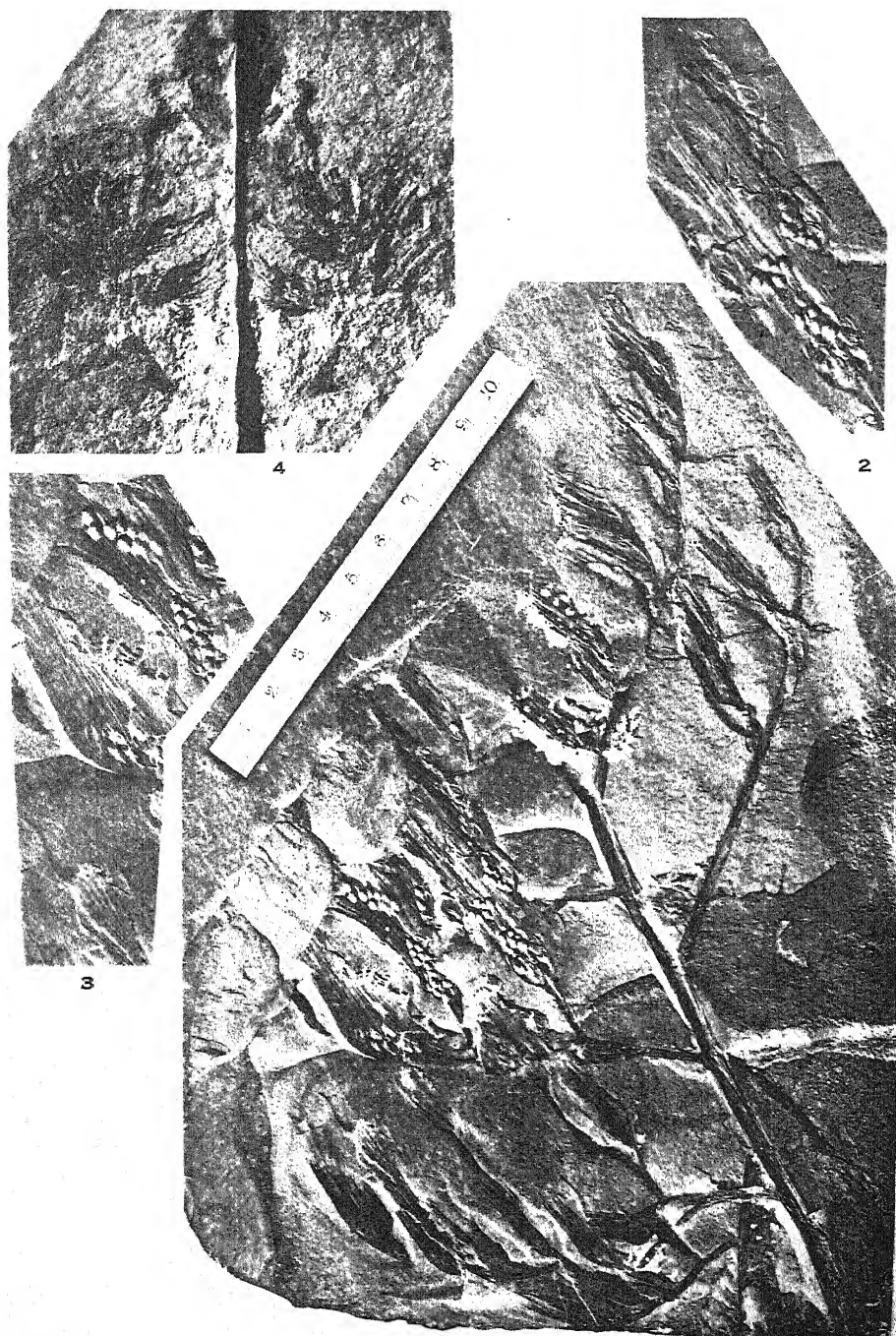
Illustrating Dr. M. Benson's paper on 'The Fructification, *Calathiops Bernhardtii*, n. sp.'

Fig. 1. A photograph of the entire specimen of *C. Bernhardtii*. Text-fig. 1 should be consulted as a key. The attached scale shows the magnification to be about $\frac{2}{3}$.

Figs. 2 and 3 represent parts of the same specimen at a slightly higher magnification. They show well the attachment and liberation of the seeds and the separation in some cases of the valvate cupular lobes. Fig. 3 shows a tuft very reminiscent of *Whittleseyia elegans* Newb.

Fig. 4. A photograph of natural size of *C. Gothani*, n. sp. This body occurs on two counter-parts and is explained by the Text-fig. 3 in which both are used.

The photograph (Fig. 4) was taken by Mr. Tams and the others by Mr. Herring at the British Museum.



1

Huth. Stubbs X. Kent.

RENSON - CALATHIOPS.

The Development of the Shoot in *Alstroemeria* and the Unit of Shoot Growth in Monocotyledons.¹

BY

J. H. PRIESTLEY, L. I. SCOTT

AND

E. C. GILLET.

(*Botany Department, The University of Leeds.*)

With eleven Figures in the Text.

CONTENTS

INTRODUCTION	161
MORPHOLOGY	162
THE COURSE OF THE BUNDLES IN THE STEM	162
DEVELOPMENT	166
THE COURSE OF THE LEAF-TRACE BUNDLES CONSIDERED FROM THE STANDPOINT OF DEVELOPMENT	170
THE GROWTH UNIT OF THE SHOOT OF <i>ALSTROEMERIA</i>	174
PHYLLOTAXIS AND ITS RELATION TO INTERNAL ANATOMY	175
SUMMARY	178
LITERATURE CITED	179

INTRODUCTION.

THE shoot system of the flowering plant is always characterized by its articulate structure, in which a series of leaves are borne at successive nodes. The attempt has recently been made to interpret this characteristic phenomenon from the standpoint of development (8) and it has led to the identification in the dicotyledon of a growth unit, which in many respects corresponds with the morphological unit of the older authors, such as the 'sprossglied' of Celakovsky (2), but which is primarily a physiological concept, since the structural limits are regarded as determined by the growth of tissues around vascular strands.

In the dicotyledon, Celakovsky identified a type of growth unit, which

¹ The substance of this paper was communicated to Section K (Botany) at the Leicester Meeting of the British Association, September 1933.

consisted of the leaf and a sectorial portion of the axis below, and which he described as mericyclic. This conception is in harmony with the single ring of vascular strands characteristic of the primary anatomy of the stem, though in whorled types it has been suggested that the upper portions of one whorl of units may completely enclose the lower portions of the units of the whorl above (3).

In monocotyledons, Celakovsky described the unit as holocyclic and conceived it as consisting of the leaf and the entire solid cylinder of the axis extending down as far as the next leaf below. The very different vascular arrangement in the monocotyledon suggests that the growth units in this group are likely to be differently constructed from those of the dicotyledon. The present study of vascular development and growth in *Alstroemeria* is an attempt to identify the growth unit in a monocotyledon shoot, which will be in keeping with its anatomy.

MORPHOLOGY

Alstroemeria aurantiaca Don., a member of the Amaryllidaceae, is a plant two to four feet in height with erect stems, which arise from a branched rhizome. It proved a convenient plant for the purpose of this investigation on account of the development of appreciable internodes and the absence of axillary buds or strongly marked nodal structure. The leaves are relatively narrow at the base, but widen out above into a lamina, asymmetric about the midrib. An interesting characteristic of the adult leaves is the twist, which always occurs at the junction of sheath and blade and causes the inversion of the lamina surface (Fig. 3). The phyllotaxis of a plant appears to be determined at the shoot apex by the number of primordia which can participate in the apical meristem simultaneously (8), the size of the primordium being approximately constant. Dissection of the bud of a growing vegetative shoot shows eight primordia crowded at the apex, and eight extending leaves in process of becoming separated from the bud by the appearance of internodes between them. The remaining leaves and internodes below these are adult. From these facts as from the arrangement of the leaves on the adult stem, a phyllotaxis of $3/8$ may be determined, in which the spiral is anticlockwise in the ascending direction.

THE COURSE OF THE BUNDLES IN THE STEM.

A transverse section of an adult internode in *Alstroemeria* shows the typical monocotyledonous structure with numerous (usually about thirty-four or more) bundles 'scattered' in a ground tissue of large-celled parenchyma (Fig. 1). Towards the periphery, the cells of the ground tissue become somewhat smaller and a band of lignified tissue separates a narrow cortex from the larger medulla in which the majority of the bundles

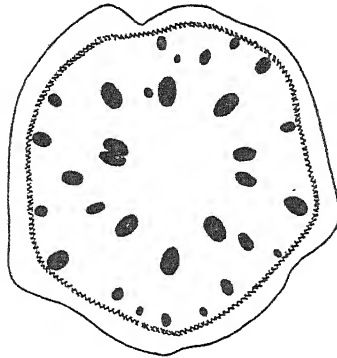


FIG. 1. Transverse section of the stem of *Alstroemeria*, shown in plan to illustrate the distribution of the bundles and the position of the zone of lignified cells. ($\times 28$).

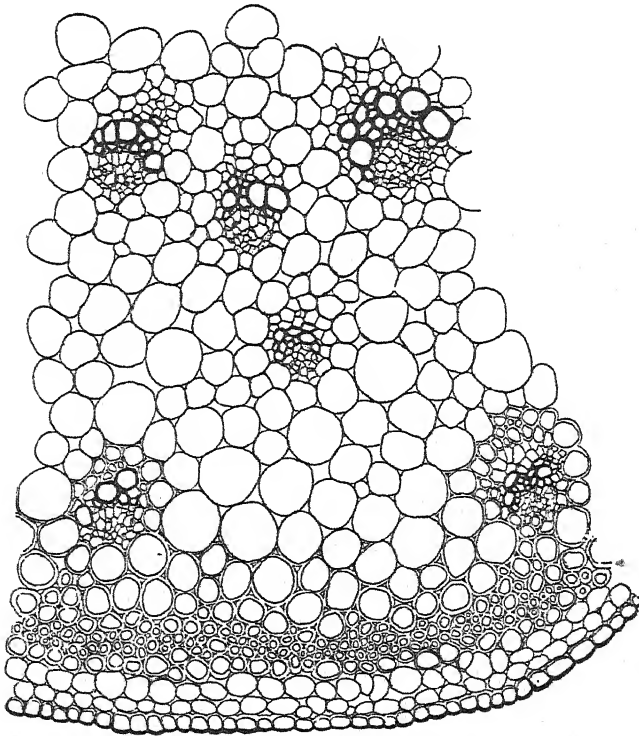


FIG. 2. Part of the transverse section of an adult internode. ($\times 200$).

are seen (Fig. 2). Although the bundles are 'scattered', these are actually as much leaf traces as the bundles in the single ring in a primary dicotyledonous stem. The course of the bundles and their connexion with the

leaves may be followed very simply by cutting a leaf across under a dilute solution of Magdala red, leaving for about one hour and then cutting a transverse section through each successive internode below and noticing in which bundles the dye is present.

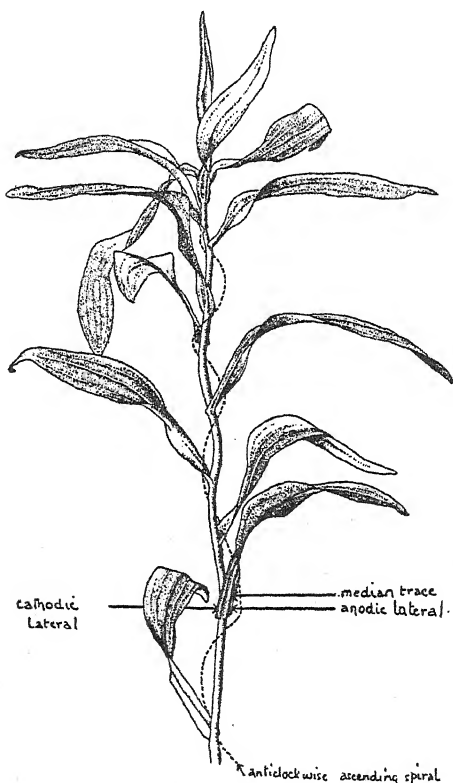


FIG. 3. A vegetative shoot of *Alstroemeria*, with annotation to explain the method of reference to lateral bundles. The anodic side is that towards which the spiral is rising. ($\times \frac{1}{2}$).

From each leaf three bundles enter the stem as the leaf trace. As the course of the two lateral bundles is different and is also related to the inversion of the lamina, it is important to have some ready means of reference to a particular side of the trace. The method adopted is that used by Nägeli ((5) loc. cit., p. 48), where the annotation of the lateral strands has reference to the direction of the phyllotaxis spiral. The side towards which the spiral is rising is described as the anodic, the other as the cathodic. Thus in *Alstroemeria*, when following the spiral upwards the cathodic side is reached first, and when the leaf insertion is turned towards the observer, the anodic lateral lies to the right of the median (Fig. 3).

The median bundle of the trace passes inwards and downwards through eight internodes and then practically vertically down through a further eight internodes as one of the eight most centrally running bundles in the stem, each of which represents the lower portion of the median

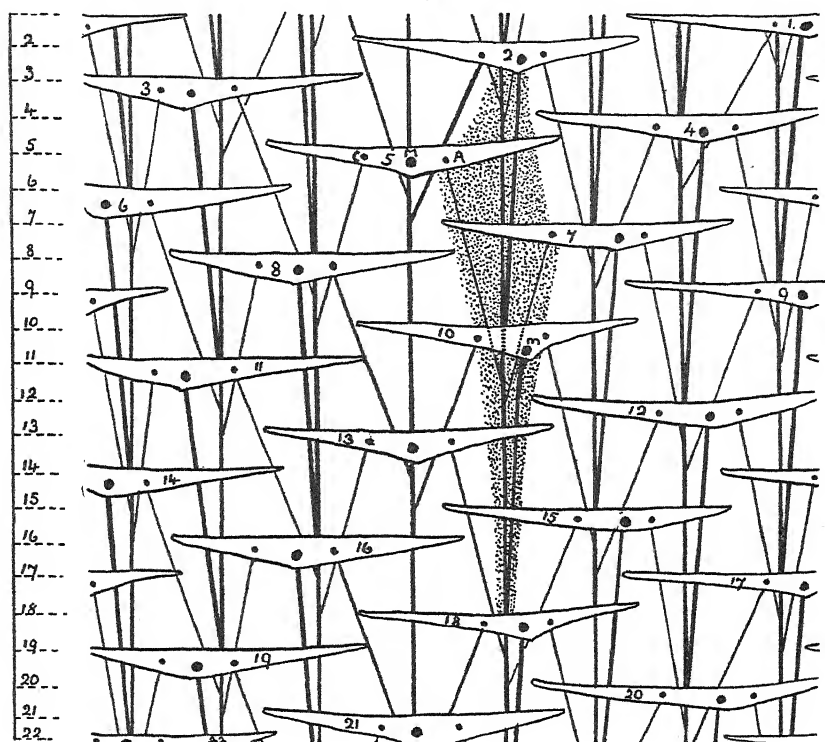


FIG. 4. Diagram to show the course of the leaf-trace bundles in the stem. The orthostichy to which special reference is made in the text is stippled. The orthostichy to the left of the stippled one is regarded as directly facing the observer, so that the foliar and axial regions of median bundles appear superposed.

bundle of one of the leaves in a single 'cycle' of the $\frac{3}{8}$ phyllotaxis spiral. Sixteen internodes below the insertion of the injected leaf, which is numbered L1, in Fig. 4, this median bundle joins that of the next leaf below on the same orthostichy, L9. The eight central bundles therefore correspond with the eight leaf orthostichies. At each node one of these bundles enters upon its oblique outward course to a leaf and is replaced in the central series by the median strand, which will eventually run into the next higher leaf on the same orthostichy. Tracing down the lateral bundles these are found to join one or other of the eight central bundles. It is interesting, however, to find that the three bundles which form the

trace of a particular leaf, may all be traced back to unions with different bundles in the central series and have no connexion with one another except when associated in the leaf. The course of the various bundles and their relation to bundles of the central system may be followed readily by reference to the diagram in Fig. 4. If, at the level of insertion of leaf 18, one considers the central bundle of the orthostichy, shown stippled in the diagram, it is seen to turn outwards and follow an upward, oblique course through eight internodes to run out as the median trace of leaf 10. As this trace turns outward at leaf 18, a new bundle replaces it in the central system. This bundle runs vertically through eight internodes and then diverges outwards to run out eight internodes higher as the median bundle of leaf 2. Whilst the median bundle of L 2 is still in the central system a strand runs out from it at about the level of L 12, to follow an oblique upward and outward course through about five internodes as the cathodic bundle of L 7. About one internode higher, another branch leaves the median of L 2 to run out through six internodes as the anodic of L 5. The level of departure of anodic and cathodic laterals from the central system varies within a few internodes, and not uncommonly both these leave the central bundle at approximately the same level and very shortly before the latter bends outwards itself. All linkages of main bundles occur in the central system; there are thus represented at any level, the main leaf-trace bundles to sixteen leaves, or two complete 'cycles' of the phyllotaxis spiral. These are seen as eight central bundles, which are the lower extremities of the median bundles of the upper eight leaves and eight median bundles at various distances from the centre of the stem as they follow their oblique upward course out to the lower eight leaves. In addition to these, numerous anodic and cathodic laterals are also represented as they pass obliquely out to the leaves.

DEVELOPMENT

The shoot apex of *Alstroemeria* consists of a dome-shaped mass of tissue surrounded by leaf primordia, eight of which are closely grouped around it without internodal separation and are still largely meristematic. The first appearance of a new primordium is a slight upfold of the superficial tissues, due in part to periclinal divisions in the dermatogen layer as well as in the underlying layers, a point which has also been drawn attention to in *Carex* by Guichard (4) and in *Dactylis* by Bugnon (1). Apart from the absence of periclinal divisions in the dermatogen, the first appearance of a primordium is similar in dicotyledons, but a difference soon becomes evident owing to the different planes of divisions of the cells in and around the young primordium. In the monocotyledons, more frequent divisions occur in the tangential plane, which add to the thickness of the

axis and the primordium and also in the radial plane. The greater frequency of these two types of division leads to the wide, encircling insertion, which is characteristic of the majority of the leaves in this group.

The meristem is four to five cells deep over the shoot apex and the first signs of vacuolation appear beneath it toward the centre. The meristematic surface layers are continuous over the eight youngest primordia, but the ninth primordium is becoming rapidly vacuolated and the next leaves are being carried away from the meristematic apex by the extension of the internodes.

A point of interest in *Alstroemeria* is the extremely early stage at which the procambial strands can be recognized in the primordia. The strand of the median vein is already visible in the youngest primordium, whilst in the second, when only 54μ long, the strands of the lateral bundles may also be recognized. Table I shows the measurements of some young primordia and the relative development of their procambial strands.

TABLE I.

The measurements give the distance from the leaf base to which the procambial strand could be recognized. Beyond this point it ended in meristem.

Length of leaf.	Length of procambial strand.		
	Median.	Cathodic.	Anodic.
1st primordium	Present	—	—
2nd " 54μ long	18μ	Present	Present
3rd primordium 90μ or 0.09 mm.	30μ	"	"
4th primordium 0.168 mm.	0.114 mm.	0.036 mm.	0.024 mm.
5th primordium 0.228 mm.	0.192 mm.	0.156 mm.	0.102 mm.

In the dicotyledon, the procambial ring is described as consisting of cells which remain meristematic when those of the pith to the inside and of the cortex to the outside vacuolate (6) and at least the two youngest primordia consist entirely of undifferentiated meristem. In *Alstroemeria*, the earlier appearance of the strands appears to be associated with a rather different manner of early differentiation. In transverse sections of young leaves, the procambial strands may be recognized by the smaller diameter of the cells and their nuclei and also the absence of nuclei from certain of the cells. The tissue mid-way between the strands and the meristematic leaf margin consists of rather wider cells, each of which is practically filled by the large nucleus. Immediately around the strands the cells are slightly larger, the nucleus remaining the same size as in the meristem cells whilst the proportion of cytoplasm increases. In longitudinal section, it is evident that the smaller diameter of the procambial cells and their nuclei is balanced by a

greater length, so that these cells and their nuclei are actually larger than those of the meristem. The procambial cells keep pace with the growth of the leaf by transverse divisions, but these are less frequent than the divisions in the cells between the strands, which remain roughly isodiametric and undergo very frequent divisions. Thus the first appearance of procambial strands in this type seems to be associated with a differentiation in the manner of growth of the cells in certain regions of the primordium, but the change appears to be an alteration in the nuclear-cytoplasmic ratio rather than in a visible commencement of vacuolation.

The procambial strands thus isolated are always in continuity with vascular tissue in the axis and differentiation in them follows early. In one case the first protophloem was recognized in the median strand of the fourth primordium from the apex and could be followed back in continuity with the phloem of more differentiated bundles. Protoxylem was first recognized with certainty in the seventh leaf. (The differentiation of xylem is probably associated with the more extensive vacuolation which occurs in about the ninth primordium.)

In the dicotyledons, the protoxylem starts as an isolated system at the base of each primordium, and this fact has been taken to have considerable bearing upon the articulate nature of the shoot (7). In *Chamaedorea* and *Cordyline*, Nägeli (5) stated that a similar isolated origin of the xylem occurred, but owing to the extremely early and rapid differentiation which seems to be a general feature of monocotyledons, it is only in these two genera that the isolated origin has been recognized with certainty. Differentiation is later in the lateral strands of the leaf and protophloem was not recognized in either of these before the eighth primordium. In a particular series of leaves, leaf 9, the youngest in the extending region, was the first in which protoxylem could be recognized in a lateral strand; this appeared in the cathodic bundle whilst the anodic only contained protophloem. In leaves 8 and 9 the first formed protophloem begins to collapse.

From the earliest stages of differentiation in the leaf, the strands of the main longitudinal veins are isolated as a continuous system, extending up from the leaf base to their union at the leaf apex. In this way five longitudinal strands appear first, of which one is the main vein, and to either side of it a subsidiary lateral and the main lateral (Fig. 5). Subsequently transverse and additional longitudinal strands appear between these in a basipetal direction.

The differentiation in the median and main laterals proceeds from the axis into the leaf, but that in the subsidiary longitudinal, which also appeared early as a procambial strand adjacent to the median, and the appearance of and differentiation in all other subsidiary strands proceeds in a basipetal direction. In one case the ninth leaf showed the strands adjacent to the median merely as procambium, whilst the twelfth had

protophloem differentiated on the cathodic side as far as the junction with the median vein low down in the leaf, whilst on the anodic side, there was a region of undifferentiated procambium between the protophloem of the basipetally differentiating bundle and that of the median vein. Rather older leaves show the presence of nine or more longitudinal veins. Two appear at each side of the leaf to the marginal side of the main lateral as the lamina grows in width and two others adjacent to the median, whilst other intermediate ones may also occur. Following these down, the one adjacent to the main lateral joins the latter before leaf insertion, whilst the more marginal one eventually dies out in the parenchyma without joining any other bundle or reaching the stem. As the vein system is gradually completed from the distal portion of the leaf towards the base, so that part ceases to grow, whilst the basal meristem of the leaf and the contiguous region of the stem continue to grow, probably at the expense of the synthesized materials transferred downwards during vascular differentiation.

In one set of sections, the behaviour of the marginal vein was different from that described above. In every third leaf, and therefore approximately on the same side of the stem, the marginal vein on the anodic side of the leaf continued its development into the stem. Here it followed a course near the periphery, outside the lignified tissue, and gradually decreased in size until, when only two or three cells in transverse section, it fused with the median vein of the third leaf below, close to the point of insertion of the latter on the stem. It is possible that this particular stem may have been taken from the periphery of a clump, where the illumination on one side of the stem was particularly favourable. In the individual bundles in the region of the bud the procambial cells show no particular seriation, and the first elements differentiated from them are therefore primary. The primary protophloem consists of similar cells, which are probably of the nature of small sieve-tubes but without companion cells. During the period of internodal extension a cambium becomes active, and for a time the radial seriation of the newly cut-off cells is a conspicuous feature of the bundles, but before extension is complete this appearance becomes less evident, owing to the differentiation of the cells into con-

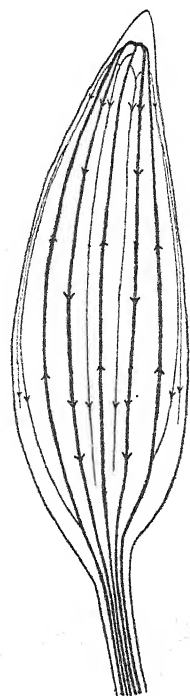


FIG. 5. Diagram showing the course of some of the longitudinal veins in the leaf. Those in heavy lines are the earlier ones to be recognized as procambial strands. The direction in which differentiation proceeds in the strands is indicated by the arrows.

spicuous sieve-tubes with small companion cells. Apart from the main vascular system which has so far been considered, there are present in the stem a few small bundles which appear to have no direct connexion with the leaves. These appear to form an anastomosing network just inside the lignified tissue, and are connected somewhat infrequently with the leaf-trace bundles. In the adult internodes they contain both xylem and phloem, but in the extending region they consist of procambium and phloem only. Followed still farther up, they decrease in size until they eventually disappear among the parenchyma cells near the region of the future lignified tissue.

THE COURSE OF THE LEAF-TRACE BUNDLES CONSIDERED FROM THE STANDPOINT OF DEVELOPMENT.

In monocotyledons the very limited nature of the cambial activity means that the primary structure is still clearly shown in adult parts and the course of the adult bundles indicates the relative positions in which the procambial strands of such bundles first appeared. Procambial strands remain meristematic when cells around them vacuolate, and as vacuolation of the central core in *Alstroemeria* is evident four to five cells beneath the apex of the meristematic dome, it is clear that any bundles running in this central part of the stem must have had their procambial strands delimited extremely early in development.

Considering the system of the main leaf-trace bundles, we have seen that at any level of the stem sixteen such bundles may be identified, and the course of a median leaf-trace bundle is vertical through eight internodes and then obliquely outwards through the next eight internodes above.

The study of the apical bud has shown us that eight young primordia are crowded around the apex, whilst eight others are in the process of rapid vacuolation and with internodes appearing between them. Although connected by meristematic surface layers, the inner tissues of the eight young primordia begin to change their manner of growth early, and consequently it is usually possible to identify the procambial strand entering the youngest primordium. As further primordia are developed, it is clear that those already present will be pushed out from the centre, and their procambial strands will be forced to assume an almost horizontal outward course. It is obviously this horizontal region of the procambial strand, which, in the adult region of the plant, is represented by that part of the course of the median trace which runs out obliquely through eight internodes to the leaf insertion. Since this outward course is initiated as soon as any younger primordia appear, it is clear that the lower part of the course of the trace, when it runs vertically through eight internodes in the central system, must have been determined so early as actually to precede

the appearance of the primordium. This is confirmed beyond doubt by consideration of the course of the leaf-trace bundles and the positions of their linkages. In Fig. 6 the strand of L1 arose in direct contact with

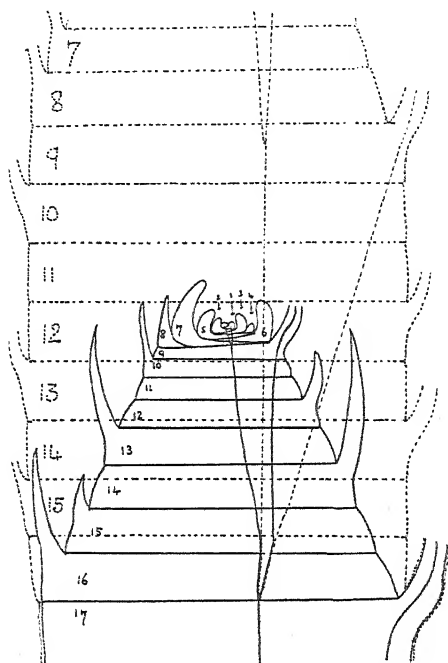


FIG. 6. Diagram showing the position of linkages of median strands in the orthostichy bearing leaves 1, 9 and 17. The diagram also shows the effect of extension of the internodes on such linkages.

that of L9 at the point where the latter began to diverge from the central system. From this point L9 travelled obliquely upwards and outwards through eight internodes, whilst through the same internodes that of L1, continued to travel in the central system. The junction of L1 and L9 has then taken place at the level of insertion of L17. Consideration will show that the only conditions which will satisfy these linkages at the levels observed is that the procambial strand of the median trace of L1 should originate when L9 was in a central position, that is when it was the youngest primordium at the apex and L17 was at practically the same horizontal level as the first leaf outside the meristematic cycle. This is a point of considerable interest, as it means that the procambial strand of L1 is actually initiated in the axis at a time when eight more primordia have to appear around the shoot apex before L1 itself appears. This period when the leaf-trace bundle is differentiating in the axis, prior to the appearance of the primordium into which it will ultimately run, is something which was not recognized in the dicotyledon in a previous paper on

its study from this point of view (8). It seems reasonable to describe this part of the leaf-trace bundle, which is represented by its course in the central region of the adult stem, as the 'axial' region. As soon as L 1 appears as the youngest primordium at the apex, this axial strand differentiates in it, and from this stage it continues to develop and to be carried outwards as a 'foliar' strand definitely associated with a visible leaf primordium.

Since the axial region of the trace of L 1 begins to be laid down as soon as the procambial strand to L 9 enters the young primordium, it is clear that the axial region of the trace to one leaf is laid down during the same period as the foliar region of the trace to the next older leaf on the same orthostichy. Thus whilst the axial region of the strand to L 1 and the foliar region of the strand to L 9 are being laid down, leaves 16-10, each with its associated internode, in turn rapidly vacuolate and extend. As these surround the axial parts of the strands destined to supply leaves 9-2, these of necessity must extend simultaneously, though the primordia 9-2 are still meristematic and the upper foliar regions of their leaf-traces are only just being laid down as procambial strands. The condition of the axial and foliar regions of the traces to supply the various cycles of leaves at a time when L 9 is the youngest primordium at the apex may best be summarized as follows:

Region.	Condition of traces in central or axial system.	Condition of traces in peripheral or foliar system.
Meristematic	Procambial strands of axial part of traces to future leaves 8-1 being differentiated just below the central dome of meristem. Primordia 8-1 not yet formed.	Primordia 16-9 relatively meristematic. Procambial strands of the foliar part of their leaf traces being laid down almost horizontally because of repeated tangential divisions in axial regions just below these primordia.
Extending	Axial regions of traces to leaves 16-9 extending. Primordia 16-9 meristematic.	Foliar regions of traces to leaves 24-17 extending.
Adult	Axial regions of traces to leaves 24-17 fully extended. Leaves 24-17 still extending.	Foliar traces to adult leaves 32-25 fully extended.

The fact that the foliar regions of one cycle surround the axial regions of the next younger cycle necessitates the simultaneous extension of these parts. This means also that unions of the strands, which involve axial strands, must originate close behind the apex since vacuolation occurs so early in this region, but that they become separated from it at an early stage by the extension which accompanies that of the foliar regions of the next older cycle of leaves, which surround this central part. This is illustrated for the median strands of one orthostichy in Fig. 6.

When the asymmetric growth of the meristematic shoot apex leads to the formation of a new primordium, tangential cell-divisions are very active

in the region underlying the new primordium, and it is largely due to this activity that the axial procambial strand is diverted outwards. That the foliar region of a median leaf-trace is a continuation of the axial region' is

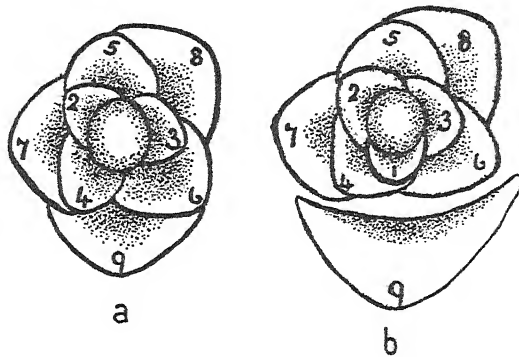


FIG. 7. Plan of the apex showing the origin of primordia. (a) shows the overlapping margins of primordia 4 and 6. (b) shows that the median region of primordium 1 is superimposed above the same sector of the stem as the margins of 4 and 6.

shown clearly by the continuity of the elements and the similarity in the degree of differentiation of this bundle before and after its divergence from the central system. When a new axial strand is first apparent as a differentiated strand distinct from the outwardly diverging median strand of the next older leaf on the same orthostichy, its degree of differentiation suggests that it is definitely younger than the strand from which it arises; this is in accordance with the view that it is to be regarded as the strand to a new primordium, which is ultimately to appear as the next on the same orthostichy.

Besides the tangential divisions, which carry out the new primordium and add to the thickness of the axis, active growth by radial divisions also occurs. By this means a primordium, which arises as a narrow upfold over a procambial strand, soon grows so as to involve a greater periphery of the apex. Consequently the surface at one horizontal level may be contributing in one part to the median region of a younger leaf, and in other parts to the more marginal regions of older leaves. Thus in Fig. 7 it may be seen that the cathodic margin of L6, the anodic margin of L4, and the median region of L1 are all derived from a common fraction of the periphery. As a result, when vacuolation occurs in the axis subtended by this periphery, it isolates, in addition to the median procambial strand to the future L1, strands to supply the margins of L6 and L4, and these both appear at this level and subsequently differentiate at approximately the same time as the axial strand to L1. The course of the adult bundles shows the linkages of both these laterals with the axial strand at approximately the same level and just prior to the bending out of the axial strand into the foliar region of its course.

THE GROWTH UNIT OF THE SHOOT OF *ALSTROEMERIA*.

The unit of shoot growth for the dicotyledon has been described as the segment of the axis which subtends a leaf initial and surrounds its leaf-trace as it differentiates (7 and 3). In *Alstroemeria* it is obviously necessary to take into consideration both regions of the course of the median leaf-trace bundle. In the upper or foliar region the strand is definitely associated with the primordium, whilst in the lower axial region the strand precedes the primordium in time of appearance. Since, however, the foliar region is a direct continuation of the axial region and the primordium appears immediately above and is entered by this strand, it seems inevitable that the whole strand, throughout both the axial and foliar regions of its course, should be regarded as the leaf-trace and the unit of shoot growth in this plant would therefore extend through sixteen internodes. In the lower region the unit will be a narrow portion of the central region of the axis, and will include one of the bundles running in the innermost ring. In the upper region the unit expands with the developing primordium, and encloses the lower regions of younger units within it. Actually in *Alstroemeria* this enclosure of inner units is only partial, but it is obvious that in many monocotyledons the upper part of the growth unit would completely encircle those within it. This concept of the single growth unit is shown in Fig. 8, and the arrangement of their foliar portions in the shoot in Fig. 9. The foliar expanded region of the unit is thus seen to extend obliquely inwards and downwards as far as the insertion of the next leaf below on the same orthostichy, and it is this region which would appear to correspond with the units figured for the dicotyledon (8). In the monocotyledon, however, owing to the very limited nature of the cambial activity, it is possible to recognize the further extension of these units downwards in the axial region, and it seems logical that this region should also be included in the complete unit of growth, since it is at the base of the axial region that the younger unit takes its origin, or is linked with the next older on that orthostichy. The recognition of the axial region of the unit is important in one respect. It makes it clear that the primordium is preceded by the procambial strand; it remains for further consideration to what extent the differentiation of the strand is causal in relation to the subsequent emergence of the primordium.

Consideration of the construction of the shoot in association with anatomy makes it difficult to accept Celakovsky's conception of the 'holocyclic' growth unit, consisting of the leaf and the entire cross-section of the shoot down as far as the next leaf below. So far as the exposed surface of the units are concerned, these would agree in the case of monocotyledons with completely ensheathing leaves, but, according to Cela-

kovsky's view, successive units are simply superposed, whilst in the present conception they fit into one another like a series of inverted cones.

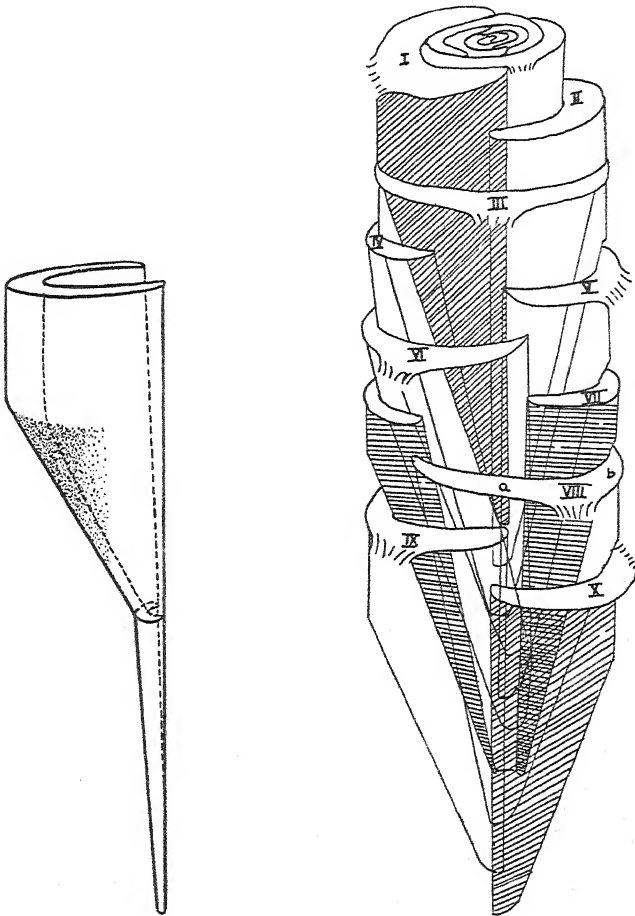


FIG. 8.

FIG. 9.

FIG. 8. Diagram showing the conception of the single unit of shoot growth in *Alstroemeria*.

FIG. 9. Diagram showing the arrangement of the foliar regions of the units of shoot growth in the stem of *Alstroemeria*.

This conception is in accord with the so-called 'scattered' bundle system of monocotyledons; these are now recognized as representing the leaf-trace systems corresponding to the various growth units as they converge towards the central region of the shoot.

PHYLLOTAXIS AND ITS RELATION TO INTERNAL ANATOMY.

A transverse section of the bud of *Alstroemeria* shows eight meristematic primordia and their arrangement enables one to recognize a $\frac{3}{8}$

phyllotaxis, leaf 9 lies on the same radius as leaf 1 (Fig. 10). The most obvious difference from a similar section of a dicotyledon is the greater overlapping of the outer leaves in the present case. The leaves are also,

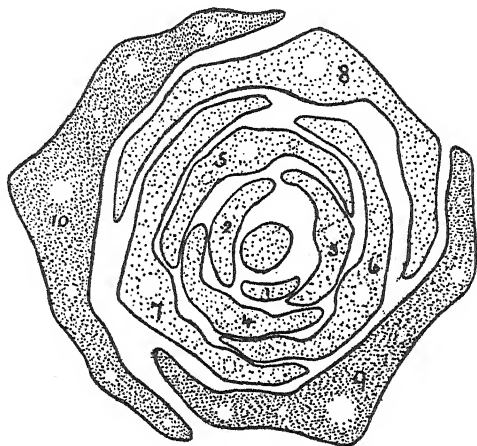


FIG. 10. The arrangement of the primordia in cross section of the bud of *Alstroemeria*. The appearance of leaf 1 on the same radius as leaf 9 agrees with a $3/8$ phyllotaxis spiral.

as shown in the figure, asymmetric in their development about the median vein, the cathodic side being more developed than the anodic. The $3/8$ system is further confirmed by the derivation of the median strand of L1 from that of L9.

From the standpoint of the derivation of one phyllotaxis spiral from another, the linkages of the lateral strands of *Alstroemeria* are suggestive. As the unit of shoot growth differentiating round a characteristic leaf-trace is approximately constant in size, it is possible that with increasing vigour of the plant the apical meristem may increase in amount, and so a larger number of units, represented at the apex by competing meristematic folds, may be accommodated simultaneously. Such being the case, the transition from $1/2$ to $1/3$ phyllotaxis will simply determine the direction of the spiral, but if in the succeeding stages we consider in each case an ascending anticlockwise spiral, it may then be predicted what the result will be if the apex continues to increase in area, so that still more primordia may be accommodated. Successive primordia seem unable to arise nearer to one another than $1/3$ of the circumference, since no simple spiral occurs, at any rate commonly, with a fraction smaller than $1/3$. If more than three competing primordia are to be arranged around the apex, it is thus clear that 1 and 2 and 2 and 3 cannot be packed any closer, but, on the other hand, they may readily be separated farther apart from one another. Consequently, to arrive at a balanced system, it is probable that the next common phyllotaxis fraction will be $2/5$, whereby primordia successive in

origin are further separated, whilst 1 and 3, separated in origin by two plastochrones, now develop closer together, as shown in Fig. 11 *a*. By this change 1, instead of being formed above 4, as in 1/3 phyllotaxis, now

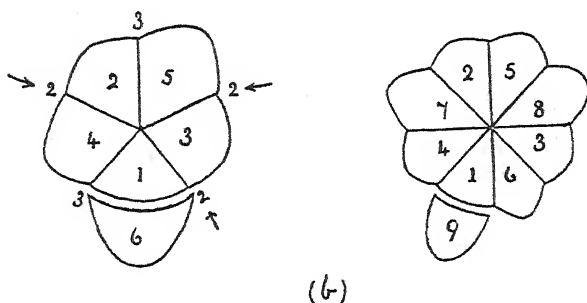
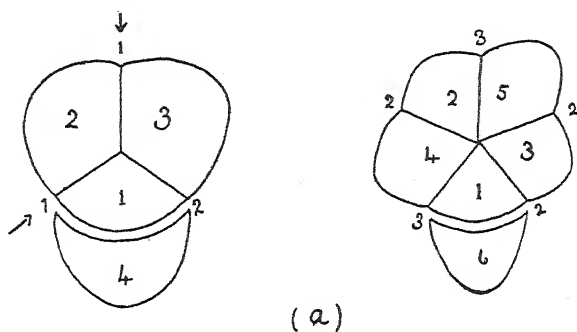


FIG. 11. (*a*) Diagram illustrating the theoretical transition from 1/3 to 2/5 phyllotaxis. The figures outside the diagram indicate the number of plastochrones separating adjacent units. (*b*) The transition from 2/5 to 3/8 illustrated in the same way.

appears above 6 and, by analogy with *Alstroemeria*, would receive its median procambial strand from that of 6, whilst 4 now lies to the left of 1. Passing on again, the same argument suggests that the next step to a stable system will introduce three more primordia, since in three cases adjacent primordia are separated in origin by two plastochrones and in two cases by three. The three former will readily separate farther from one another, whilst the two latter are still able to develop if closer approximated, since the competition between them is not so active. Fig. 11 *b* shows that 6, which in 2/5 phyllotaxis was below 1, now lies to the right, so that in the change from 1/3 to 2/5 the shift in position of 1 was to the right and in the change from 2/5 to 3/8 to the left. In 3/8, as the course of the bundles in *Alstroemeria* has shown, the median bundle of

L1 takes its origin from that of L9, but it is interesting to find that the previous linkages of '1' in the theoretical transitions, discussed above, are still suggested by the linkages of the median of L1 with the cathodic lateral of L4 and the anodic lateral of L6.

SUMMARY.

The present study of the development and anatomy of *Alstroemeria* is an attempt to identify a unit of shoot growth for a monocotyledon, which will be in keeping with its anatomy.

The course of the bundles shows the leaf-trace to consist of three bundles. The median bundle may be followed obliquely inwards and downwards through eight internodes and then down a further eight in a vertical direction near the centre of the axis, where it finally takes its origin from the ninth leaf below, thus giving confirmation to the $3/8$ phyllotaxis, which may also be recognized on morphological grounds. The recognition of the median leaf-trace bundle, sixteen internodes below the insertion of the leaf, is shown to mean that the procambial strand to leaf 1 must be laid down at the time when leaf 9 is the youngest primordium so far developed. Consequently the course of the median leaf-trace bundle through the eight lower internodes must be laid down prior to the appearance of the associated primordium, and is described as the axial region. As soon as the primordium appears the axial strand differentiates into it, and from now on continues to differentiate in an obliquely outward direction, as the primordium is carried outward from its central position by active tangential divisions beneath it. The region of the leaf-trace laid down in the axis after the appearance of the primordium is described as the foliar region.

In the dicotyledon the unit of shoot growth has been described as the segment of the axis, which subtends a leaf initial and surrounds its leaf-trace as it differentiates. Applying this conception to *Alstroemeria* the unit must be considered to extend through sixteen internodes. In the axial region it will presumably be narrow and surround a single axial strand, whilst in the foliar region it expands with the growth of the associated primordium and in its upper regions surrounds three strands. The lateral leaf-trace strands also show linkages with bundles in the central system of the stem, but the three bundles of one trace all arise from different axial bundles, all of which supply the median bundles of different orthostichies of leaves. An example of such linkages is that of the adjacent laterals of leaf 6 and leaf 4 with the axial region of the median of leaf 1. The interest of these linkages is discussed from the point of view of the derivation of the $3/8$ spiral by successive steps from $1/3$ through the $2/5$.

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Studies in *Coprinus sphaerosporus*.

II. The Inheritance of Various Morphological and Physiological Characters.

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With Plate VI and fourteen Figures in the Text.

CONTENTS.

	PAGE
1. INTRODUCTION	181
2. EXPERIMENTAL PROCEDURE	182
3. GENERAL OBSERVATIONS ON FERTILITY AND THE PRODUCTION OF DIPLOIDS	186
4. SPORE GERMINATION AND GROWTH	188
5. SEGREGATION IN VARIOUS CROSSES	189
(a) 30×17	189
(b) 16×15	189
(c) 21×10 and 10×21	189
(d) 10×2	191
(e) $21/10 \times 2$	191
(f) $30/21/10 \times 2$	192
(g) 30×21 and 21×30	192
(h) 30×2	192
(i) $21/30 \times 2$	192
6. DISCUSSION	197
7. SUMMARY	202
LITERATURE CITED	204

I. INTRODUCTION.

IN a previous paper (3) the pairing properties of various haploid and diploid strains of *Coprinus sphaerosporus* were determined, and in the present account further pairings have been made and the reactions of the different strains on one another recorded. Some of the original diploid strains have now been in culture for upwards of two years and observations on their fertility over this period are included in the present account.

The major part of the present paper is devoted to a study of the inheritance of the numerous morphological and physiological differences which exist between the various haploid strains. It has involved the production of some 8,000 cultures and a determination of the growth-rate in some 3,500 cases. No attempt has been made to work out the inheritance of the sex or incompatibility factors involved. The results obtained in working out the inheritance of the morphological and physiological factors have proved useful in throwing some light on the transference of nuclei from a diploid to a haploid mycelium when the latter is being diploidized by the former.

On the experimental side an account is given of a method, developed for use in these experiments, by which growth-rates can be determined in test-tube cultures in place of the more usual Petri dishes. This has led to a great reduction in the percentage of contaminated cultures which, if at all high, involves a great waste of both time and labour.

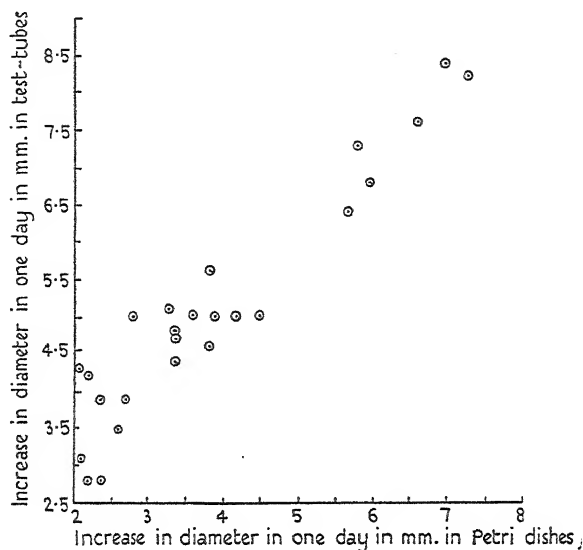
2. EXPERIMENTAL PROCEDURE.

The original diploid strain *Coprinus sphaerosporus* is called strain A and haploids derived from it are referred to by numbers as described in the previous paper of this series. The haploids used for all pairings in the present work are derived from spores of the diploid 5×3 , and they are in turn referred to simply by numbers. Where it is necessary to distinguish between the two haploid generations those derived from strain A will have the letter B prefixed and the succeeding generation the letter C. The scheme of reference to the diploids resulting from the union of two haploids, and to a diploid produced by the diploidization of a haploid by another diploid is the same as in the previous paper.

To determine the most suitable medium on which to measure growth-rates and which would at the same time show up the morphological differences between the various haploids, three media were made up, namely, Richard's solution agar, 1.5 per cent. malt agar, and plain agar. Thirty haploids from fruit-bodies of strain 5×3 were grown on Petri dishes containing these media and their growth-rates determined. Of the three media, malt agar proved the most suitable from the point of view of differentiating the various mycelial types. All the strains grew more rapidly on plain agar and less rapidly on Richard's solution agar than they did on malt. The difference in rate between the fastest and slowest strains on each medium was, however, slightly greater on malt than on either of the other media, and for this reason, combined with its suitability from the morphological point of view, it was adopted for all further work.

Where such large numbers of cultures were necessary, as in the present case, it was found impossible to use Petri dishes for growth-rate deter-

minations owing to the big percentage of contaminated cultures always present. An experiment was therefore set up to compare the rates of growth of various strains in test-tubes and Petri dishes. The medium for

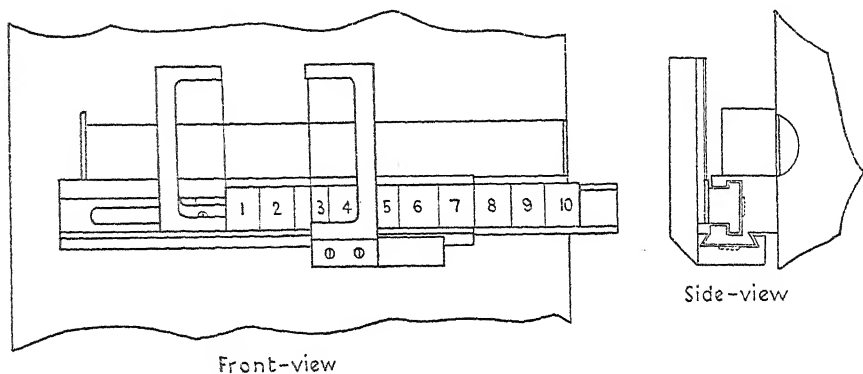


TEXT-FIG. 1. For explanation see text.

all the cultures was prepared in one lot, and each batch of test-tubes and Petri dishes was sterilized for the same length of time. The Petri dishes used were 9 cm. in diameter and the test-tubes were 15 cm. long with an internal diameter of 18 mm. A dent was made in the wall of each test-tube 3 cm. from the open end in such a way that on putting medium into the tube and laying it horizontally with the dent downwards, a column of medium of even thickness was formed, the medium about half filling the tube up to the dent. Twenty-five strains were used in the experiment and it was carried out in quintuplicate. Text-fig. 1 shows the relation between the growth-rates of the various strains in test-tubes and Petri dishes to be approximately a straight line. The strains which deviate most from the mean varied greatly in appearance, and such deviations could not be accounted for on morphological grounds. It thus appears that the range of growth-rates in test-tubes is much the same as that in Petri dishes for the cultures used in this experiment. At the same time it will be seen that the growth-rates of all the strains are greater in test-tubes than in Petri dishes, which, since the morphological characters are not appreciably affected, is advantageous in that the effect of errors in measurement over the same period of time is reduced.

In order to increase the accuracy of measurement in determining

growth-rates an instrument of the bifilar micrometer type was designed suitable for measuring the diameter of a fungal colony along the long axis of a test-tube. It is shown in front and side-view in Text-fig. 2. In operation a test-tube was inserted from the right above the scale, and behind the pairs of cross-wires, until its closed end reached the stop shown



TEXT-FIG. 2. Front and side-view drawings showing the construction of the bifilar micrometer used in measuring growth-rates.

on the left in front-view. In this position it fitted into the hollow cut in the metal back on which the micrometer was mounted, the rim and cotton-wool plug of the test-tube projecting beyond the support to the right, so that it lay parallel to the scale. The whole apparatus was mounted at an angle of 45° to the horizontal. The test-tube was now rotated until light from an overhead lamp was seen reflected from the agar. The two pairs of cross-wires were then adjusted one over each end of the colony, when a direct reading of the length of the colony was obtained on the scale. Taking readings by reflected light, was found to be much more accurate than by transmitted light particularly when the colony produced only a small amount of aerial mycelium.

Single-spore colonies were obtained by the spore-suspension method as described in the preceding paper. It was found, however, in the case of spores from a just-ripened fruit-body that approximately 100 per cent. germination was obtained in sixteen to twenty-four hours, and that this was the case with all the strains tested. It was therefore possible to modify the method previously adopted, and while securing as representative a sample of colonies as before, to reduce appreciably the labour involved. Instead, therefore, of making a dilute spore-suspension as previously described, and transferring the colonies as they became apparent to the naked eye, a stronger suspension was prepared, and after twenty-four hours had been allowed for germination the culture was examined under the microscope and the colonies were removed successively as they appeared

in the field. The advantage of the modified technique was that any number of single-spore colonies which had been transferred constituted a representative sample, whereas in the original method, due to delay in the germination of certain spores (possibly bearing a particular group of factors) and differences in the growth-rates of the various colonies, a sample typical of the whole was only obtained when all the colonies on each plate had been collected. In each cross 300 single-spore colonies were transferred to malt agar slopes. In some cases most of the colonies continued their growth, whereas in others only a varying percentage developed normally or at all (*vide* section 4). When the percentage was too small (the aim being to obtain 200-250 normal colonies) a fresh spore-suspension was prepared and additional colonies transferred to make up the required number.

TABLE I.

30 × 17	16 × 15	21 × 10 10 < 21	21/10 × 2	10 × 2	30/21/10 × 2	30 × 21 21 × 30	21/30 × 2	30 × 2
a 28	a 23							
b 5								
c 12								
d } 55	d } 33	d 19 } 43	d 13 } 44	d 25 } 40	d } 87	d 4 } 90	d 9 } 96	d 11 } 59
e } 55	e } 33	e 24 } 43	e 31 } 44	e 15 } 40	e } 87	e 86 } 90	e 87 } 96	e 48 } 59
f 1				f 5	f 0.5		f 1	f 2
		g 8	g 10		g 1			
		h 22	h 11	[h 6]				
	i 25							
						j 2		
						k 2		
						l 2		
						[m 1]		
		n 19	n 21		n 9			
							o 0.5	o 8
					p 0.5		p 1	p 31
(q) 15								
	s 3					(r) 3	r 0.5	
		t 7	t 3					
				u 4				
				v 6				
				w 0.5				
				w 3				
				x 23				
				x 9				
				[y 5]				
					[z 2]			
				[β 8]			a 1	

The single-spore colonies were allowed to grow for at least ten days on the malt agar slopes when inocula of as nearly equal size as possible were transferred to the dented test-tubes. The growth-rates in the twenty-five haploid C strains were determined from day to day when making a comparison between the growth-rates in test-tubes and Petri dishes, and it was found that in all strains they were approximately constant from the

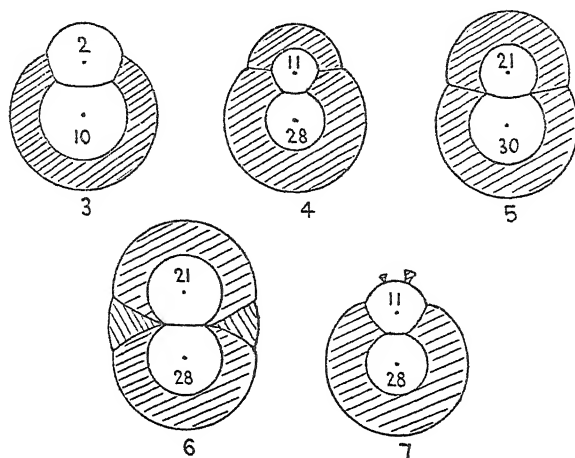
second day to the seventh. Colonies in the dented tubes were accordingly measured on the second and the seventh day from the date of inoculation, and their growth-rates as recorded are the differences between these two readings. Cultures were first made in duplicate and the growth-rate in each case was taken as the mean of the two differences. This was found, however, to be unsuccessful in effectively reducing the deviations from the mean, and as it was not practicable to increase the number of cultures of each strain further, the practice of culturing the individual strains in duplicate was dropped in ensuing experiments. On the seventh day, after measurements had been taken, all the colonies were separated into groups on a morphological basis, and the group into which each colony fell was recorded opposite its growth rate. Frequency distributions of the various groups were then prepared.

3. GENERAL OBSERVATIONS ON FERTILITY AND THE PRODUCTION OF DIPLOIDS.

Strain A has now been in culture for more than two years during which time mycelial inocula have always been used in preparing subcultures. When originally obtained, and for about a year afterwards, it fruited freely on malt agar slopes, the fruit-bodies being in most cases perfect. Subsequently it was found that the number of fructifications produced was decreasing, and that many never reached maturity. The same phenomenon has been observed in the generation of diploids derived from the haploids produced by strain A. Originally fruiting freely they are now in nearly all cases almost sterile, and only rarely give rise to fruit-bodies and then seldom to perfect ones. A comparison of the fertility of the B and C haploids when paired also reveals a decrease. The C haploids were paired amongst themselves in order to ascertain the sex of the different strains, and were found to fall into two sex groups as was the case with the B haploids. Out of a total of 99 inter-sex pairings only 11 per cent. produced perfect fruit-bodies, whereas in the case of 225 similar crosses between the haploids of the B generation 24 per cent. produced perfect fructifications.

Following the determination of the sex of the C haploids, inocula from selected pairs of them were taken and placed near one another in Petri dishes with the object of obtaining diploids, the origin of which from one or other member of each pair was known. The effects of the different members of each pair on one another were noted, and diagrams showing typical examples of the different types are given in Text-figs. 3-7. The dimensions given in these figures are only approximately correct and aim merely at giving a general impression of the reactions of the strains. All diploid mycelia are shaded, and the numbers are those of the haploid strains concerned. Text-fig. 3 shows the result of pairing strains 2 and 10, strain 10 has been diploidized relatively early, but 2 has remained haploid.

This cross has been repeated several times, always with the same result. On pairing strain 2 with strain 30 a similar reaction was obtained in that 2 again remained haploid. The only case previously observed in which



TEXT-FIGS. 3-7. For explanation see text.

one strain of a pair remained haploid while the other was diploidized was in the cross between B₃ and the saltant B₅, 7, in which the saltant continued haploid. In the account previously given (3) of the reactions of two strains of opposite sex it was stated that in the second member of a pair to become diploidized the diploid mycelium usually appeared at a few scattered points on the periphery. This has again been found to be the case as shown in Text-fig. 7 where strain 28 became diploidized early, and 11 gave rise to a diploid mycelium at two points only. It has also been found, however, with the present strains that both mycelia may become diploidized uniformly all round their peripheries, even when one becomes diploid some time after the other. This was found to be the case in several crosses, e.g. 21 × 30, Text-fig. 5, and in another pairing of 11 × 28, Text-fig. 4. The reaction shown in Text-fig. 6 between strains 21 and 28 has been observed several times. In this case the two strains were approximately equal in growth-rate and became diploidized simultaneously. It differs from the other types in that the two diploid mycelia are separated by wedges of a dense white and fluffy mycelium. Whether this mycelium is diploid or not was not determined.

As previously recorded, it was found that a haploid could be diploidized by contact with a diploid derived from the pairing of itself with another diploid. It has now been established in all the cases examined that a haploid can be diploidized by a diploid derived from two different haploids, and in each instance has been found to be fertile.

4. SPORE GERMINATION AND GROWTH.

As already stated, spores from a just-ripened fruit-body gave about 100 per cent. germination on malt agar within twenty-four hours, whereas spores which had been allowed to remain for several days after reaching maturity were not only slower in germinating, but a number of them were not found to germinate even after several days.

When germinated spores from the various crosses were transferred to malt agar slopes it was found that only a certain number continued to grow in each case. The minimum percentage which continued to grow was 50 per cent. in the spores from the cross 15×16 and a maximum of 84 per cent. was obtained in the cross 30×2 . In the other crosses the number varied from 60 to 80 per cent. respectively. It is not probable that cessation of growth was due to heating of the colony by the inoculating needle as the latter was always allowed to cool before being used, and only rarely was the germinating spore lifted direct on the point of the needle, the normal practice being to cut out the agar round the spore and transfer the two together. A small number of the colonies which appeared not to grow could be accounted for if they had not in effect been transferred but had slipped off the agar as it was lifted. Such a possibility is, however, small, as an examination of some fifty blocks of agar which had been transferred, but which had produced no apparent growth after several days, revealed the presence of a colony in each case. Colonies on being transferred consisted of a spore with at most two short hyphae growing from it, one or both of which had occasionally branched. An examination of the fifty cultures to which reference has already been made showed a very small and much branched colony in each case, it is therefore evident that growth continued for some time after the transfer had been made. In only two instances did colonies such as these become visible to the naked eye even after several weeks incubation, and in neither of these cases did the diameter exceed 1.5 mm. A dilute suspension of spores from the diploid 15×16 was prepared, poured over medium in the ordinary way, and after a week's incubation a search was made in the areas between the visible colonies to discover if any dwarf colonies were present. Dwarf colonies were found in large numbers, and were similar to those in the transferred agar blocks. They were all of much the same size and characterized by the much branched form of the mycelium. From these observations it appears that while practically all the spores are capable of germination if sown immediately they ripen, a certain percentage have only a limited growth and give rise to microscopic colonies. Whether the percentage is a constant for the spores from a particular cross was not determined. Two estimates on spores from the cross 15×16 have, however, been made, based respectively on 396 and 389 transferred colonies, and in each case 51 per cent.

normal and 49 per cent. dwarfs were obtained. The appearance of the dwarf colonies was similar to that of colonies produced by certain X-rayed ascospore colonies of *Chaetomium cochliodes* (2, p. 399), though in the latter case growth ceased earlier than it did in the case of the basidiospores.

5. SEGREGATION IN VARIOUS CROSSES.

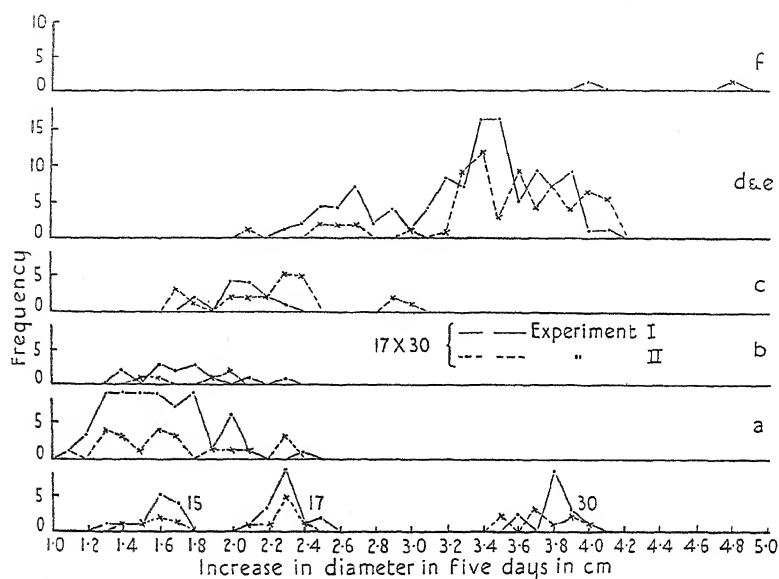
When all the segregants had been obtained, representatives of all the types from every cross were grown together and compared, and a reference letter given to each morphological type. A comparison was then made between the growth-rates of each type in the various crosses from which it was obtained, and as can be seen from the graphs, these were found to be very similar in each case. Altogether twenty-eight types were obtained and a description of them is given at the end of this section. In the Text-figs. referred to below the frequency distributions of the parental strains (and in most cases of one or more other C strains in addition) are given at the bottom of each graph, the frequency curves of the segregants being found above these.

(a) 30×17 . The experiment was repeated twice, and in each case the various segregants were grown in duplicate. Five types were obtained in the F_1 generation (Text-fig. 8 and Pl. VI, Fig. 1), the numbers of each type expressed as a percentage of the whole in each of the two experiments being as follows: type a, 24 and 32%; b, 4 and 6%; c, 17 and 6%; d and e, 54 and 55%; and f, 1 and 1% (when the percentage worked out at less than one it was taken to the nearest half per cent., when greater than one to the nearest whole number). It will be seen that the relative frequencies agree reasonably well in the two experiments when due allowance has been made for the small numbers of each type available. In the two experiments respectively 132 and 200 segregants were available for measurement when contaminated cultures and abnormal colonies had been discarded.

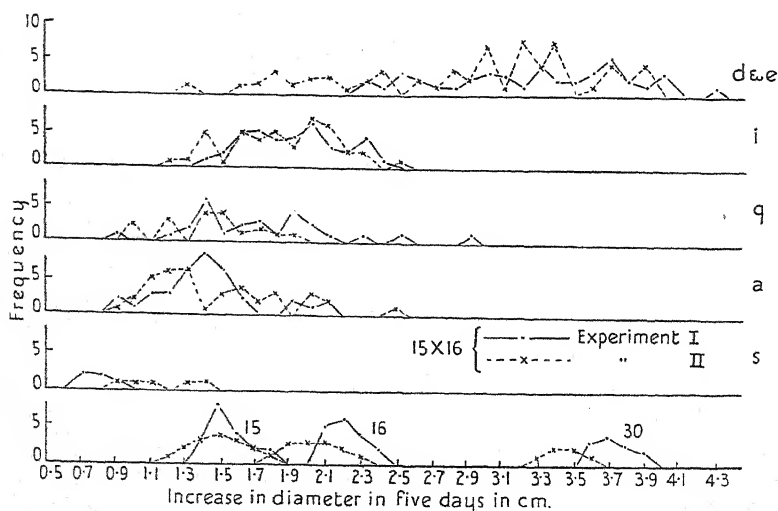
(b) 16×15 . This cross was also repeated twice and the colonies measured in duplicate. The numbers of available colonies were respectively 149 and 176, and again five segregant types were obtained (Text-fig. 9 and Pl. VI, Fig. 2) whose relative frequencies in the two experiments were as follows: type s, 3 and 3%; a, 22 and 23%; q, 19 and 12%; i, 26 and 24%; d and e, 29 and 37%. While the agreement between the frequencies of the different types in the two experiments is better than in the cross 30×17 the range of growth-rates of the types q, and d, and e, differs considerably.

(c) 21×10 and 10×21 . In these reciprocal crosses 203 and 206 colonies were respectively available for measurement. In each case six types were obtained (Text-fig. 10 and Pl. VI, Fig. 3), each type from one cross being identical with a type from the reciprocal cross. The

percentage frequencies of the types from the crosses 21×10 and 10×21 were respectively as follows: type g, 9 and 7%; h, 30 and 14%; t, 11 and 4%;



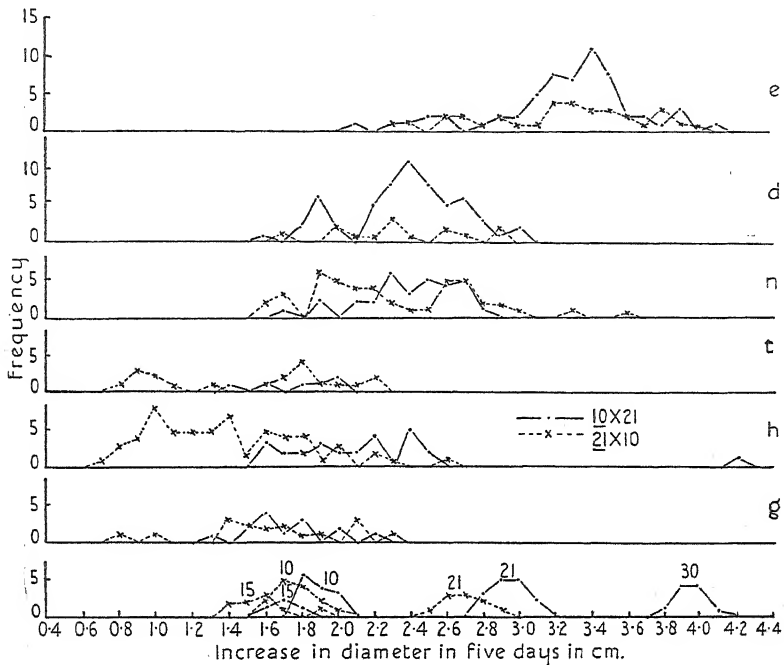
TEXT-FIG. 8. For explanation see text.



TEXT-FIG. 9. For explanation see text.

n, 23 and 15%; d, 10 and 20%, and e, 17 and 31%. The agreement between the relative frequencies of the six types in the reciprocal crosses is not as

good as that in the duplicate experiments of the preceding crosses, nor is the respective range of growth-rates in strains h and t exactly comparable, but without a large increase in the data available it cannot be said that



TEXT-FIG. 10. For explanation see text.

there is any real difference, either in the frequency or growth-rate of the individual types from the reciprocal crosses.

(d) 10×2 . Ten segregating types (Text-fig. 11 and Pl. VI, Fig. 4), found in the 154 colonies available for examination, were obtained from this cross. Their percentage frequencies were as follows: type h, 6%; β , 8%; y, 5%; x, 23%; d, 25%; e, 15%; v, 6%; u, 4%; f, 5%; and w, 3%.

(e) $21/10 \times 2$. In this instance where the haploid 21 was diploidized by the diploid 10×2 , 217 colonies were available and were found to fall into nine different types (Text-fig. 11). The relative frequencies of the various types were as follows: type g, 10%; h, 11%; t, 3%; x, 9%; n, 21%; d, 13%; e, 31%; v, 0.5%; and w, 0.5%. Certain of these types, namely, h, x, d, e, v, and w, were segregants both of 10×2 and $21/10 \times 2$. The frequency distributions of both groups are shown in Text-fig. 11, and it will be seen that the growth-rates of those strains derived from the different parents, and which were considered identical morphologically, are on the whole very similar to one another. Other strains, namely, β , y, u, and f, from 10×2 , and g, t, and n, from $21/10 \times 2$ were not found to have a

counterpart in the segregants from the other cross. In Table I, in which are set out the segregants of each cross and their percentage frequencies, it will be seen that all the types from the cross $21/10 \times 2$ which are not represented in the 10×2 segregants are to be found among those from the 21×10 cross, and a comparison of their growth-rates in the graphs in Text-figs. 10 and 11 show them to be very similar. At the same time certain types from the 10×2 cross were not found in the $21/10 \times 2$ (nor in the 21×10) segregants. These strains are f, u, y, and β , and it will be seen that their frequencies (5, 4, 5, and 8%, respectively) are relatively small. The strains d, e, and h, are the only ones common to all three crosses.

(f) $30/21/10 \times 2$. In a total of 186 colonies six types were found (Text-fig. 14). Their frequencies were as follows: type n, 9%; g, 1%; p, 0.5%; z, 2%; f, 0.5%; and d and e together, 87%.

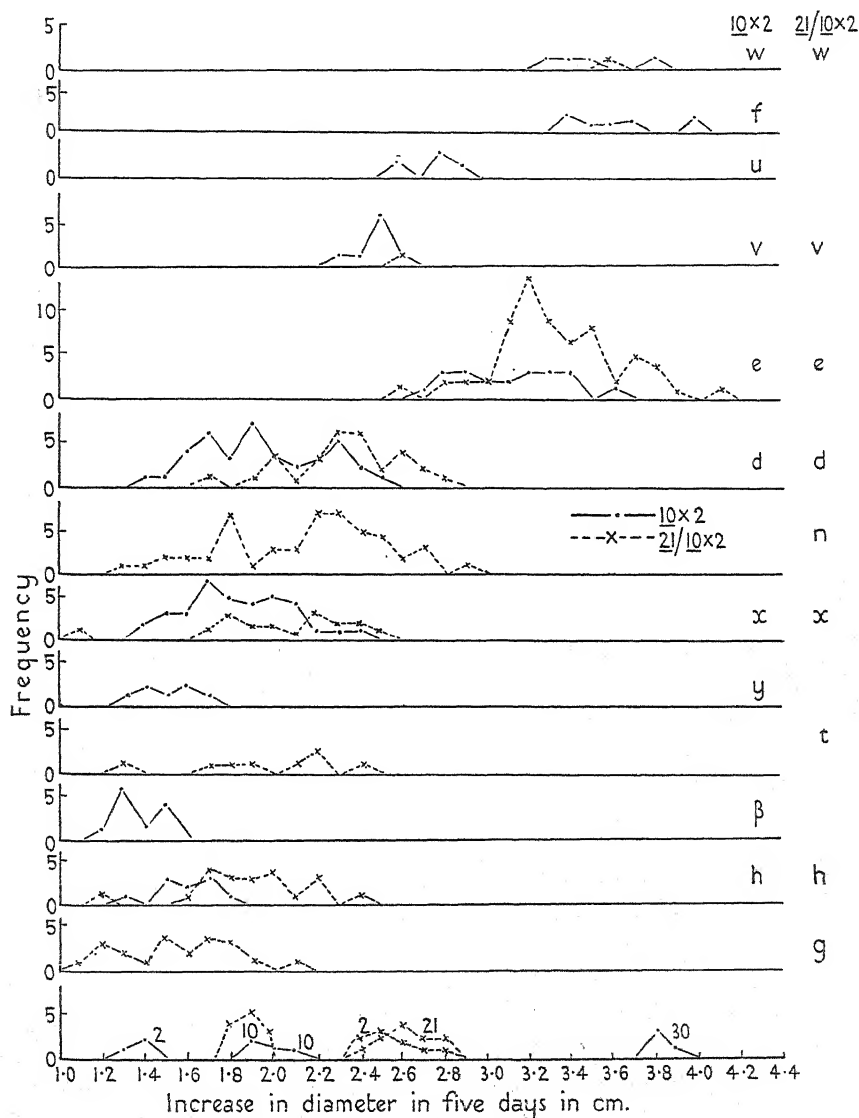
(g) 30×21 and 21×30 . In these reciprocal crosses (Text-fig. 12 and Pl. VI, Fig. 5) 130 and 101 colonies were respectively available. The cross 30×21 gave six and 21×30 five types. The latter were morphologically identical with five of the 30×21 types, and as the remaining type m only occurred with a frequency of 2%, it is not improbable that should a further sample be taken from the cross 21×30 it would be obtained in that as well. The remaining five types occurred in the reciprocals 30×21 and 21×30 with the following percentage frequencies: type j, 2 and 2%; k, 1 and 3%; r, 1 and 6%; l, 1 and 4%; d, 3 and 4%; and e, 92 and 80%. The agreement is not good in most instances, but the frequencies are very small in all cases except type e, so that, as was the case with the reciprocal crosses 21×10 and 10×21 there is no reason to believe that a real difference between the two exists. The growth-rates agree fairly well, except for strains j and k.

(h) 30×2 . 246 colonies were measured in this cross and fell into five morphological groups (Text-fig. 13 and Pl. VI, Fig. 7) which occurred with the following frequencies: type p, 31%; o, 8%; d, 11%; e, 48%; and f, 2%.

(i) $21/30 \times 2$. 214 colonies were available for measurement and belonged to seven morphological types (Text-fig. 13 and Pl. VI, Fig. 6). The frequencies of these were as follows: type p, 1%; α , 1%; o, 0.5%; r, 0.5%; d, 9%; e, 87%; and f, 1%. It will be seen that all these types with the exception of α are represented in the segregants of one or other of the crosses 30×2 or 30×21 . Types d and e are common to all three crosses, types f, o, and p, to $21/30 \times 2$ and 30×2 , and type r to $21/30 \times 2$ and 21×30 . j, k, l, and m, all of which occur infrequently, are only found in the cross 21×30 . A comparison of the growth-rates of the various types taken in turn which are common to different crosses reveals a satisfactory agreement in each case.

In Table I the five types in square brackets—h, y, β , z, and m—are

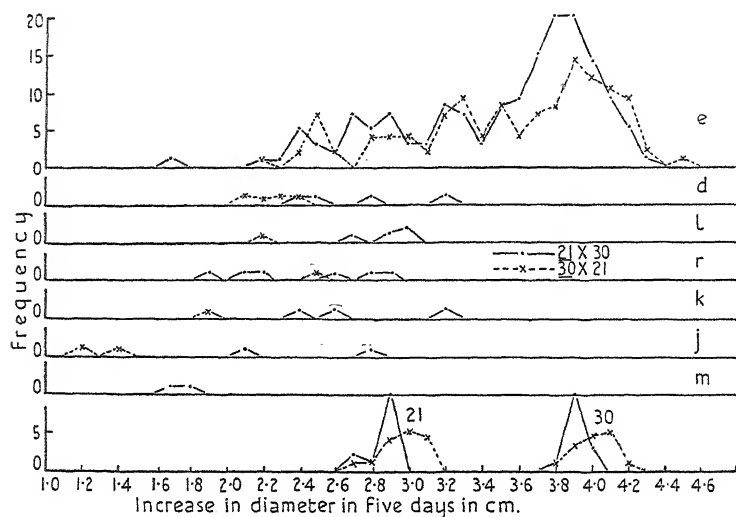
not represented in the photographs in the Plate. The two types in round brackets—q, and r (of 30×21)—were found to have altered in appearance



TEXT-FIG. 11. For explanation see text.

between the time of their first examination and when all the types were compared some months later. In the columns 21×10 and 30×21 the frequencies quoted are the means of those of the reciprocal crosses in each case.

Altogether twenty-eight types were found in the segregants of the various crosses made in these experiments. A morphological description of the various types and also of their parents is given below.



TEXT-FIG. 12. For explanation see text.

Strain C 30. Pl. VI, Figs. 1, 5, 6, and 7. The aerial mycelium is very freely produced, fairly dense, and is evenly disposed over the surface of the colony.

Strain C 17. Pl. VI, Fig. 1. Aerial mycelium plentiful and loose in texture, distribution over the surface of the colony even.

Strain C 16. Pl. VI, Fig. 2. Aerial mycelium fairly plentiful, loose in texture and evenly distributed.

Strain C 15. Pl. VI, Fig. 2. Aerial mycelium rather thin, not dense in texture and showing patches of thicker and thinner mycelium over the surface.

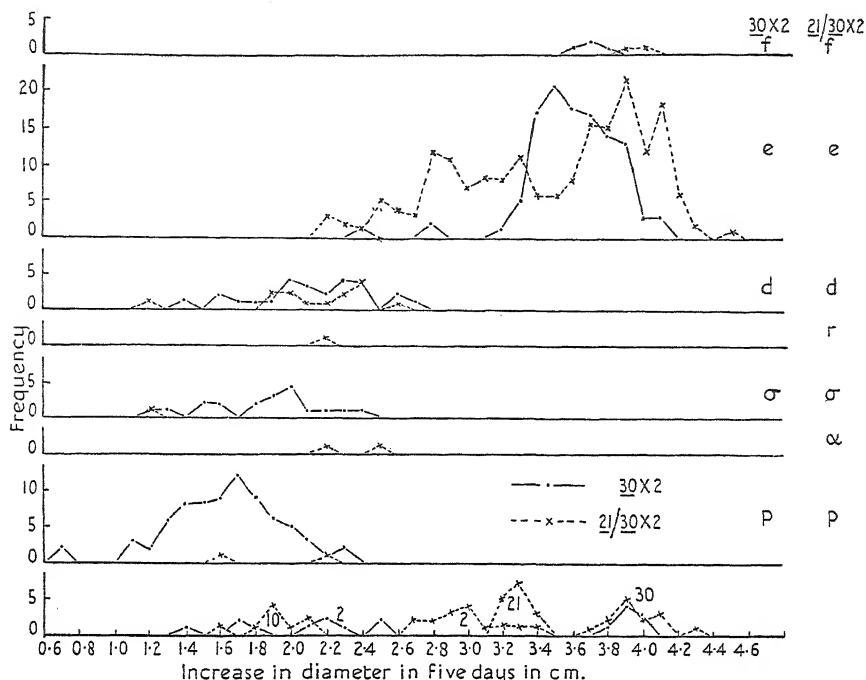
Strain C 21. Pl. VI, Figs. 3, 5, and 6. Aerial mycelium very plentiful, loose in texture and evenly distributed.

Strain C 10. Pl. VI, Fig. 3. Aerial mycelium thin, loose in texture and evenly distributed.

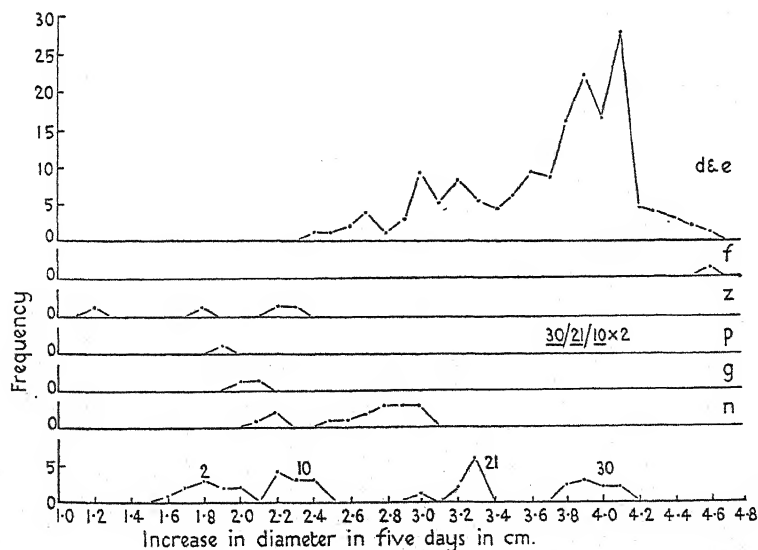
Strain C 2. Pl. VI, Figs. 6 and 7. Aerial mycelium very thin except at the centre of the colony where it is a little thicker.

Type a. Pl. VI, Figs. 1 and 2. Aerial mycelium plentiful but dense and lying very close to the surface of the medium. Distribution of the mycelium over the surface of the colony even, gradually thinning out towards the periphery.

Type b. Pl. VI, Fig. 1. Aerial mycelium plentiful and loose in



TEXT-FIG. 13. For explanation see text.



TEXT-FIG. 14. For explanation see text.

texture at the periphery of the colony. At the centre the mycelium is in thin rhizomorph-like strands.

Type c. Pl. VI, Fig. 1. Aerial mycelium plentiful and loose in texture at the periphery. At the centre of the colony it consists of numerous rhizomorph-like strands.

Types d and e. Pl. VI, Figs. 1-7. In these two types the mycelium is very plentifully produced. In type e the whole surface of the colony is evenly covered, but in type d around the inoculum there is in general an area of relatively thin mycelium. These two types were found to grade into one another to some extent, and only in the segregants of some crosses was it possible to separate them into two groups. For this reason their frequencies have, for the purpose of comparison, been added together in all cases in compiling Table I.

Type f. Pl. VI, Figs. 1, 4, 6, and 7. Aerial mycelium moderately plentiful, loose in texture and 'granular' in appearance. Radiating rhizomorph-like threads were present around the inocula in some cases, but were not strongly marked.

Type g. Pl. VI, Fig. 3. The mycelium was extremely thin so that the colony could scarcely be seen by transmitted light.

Type h. Pl. VI, Fig. 3. Aerial mycelium very thin, but loose in texture. Distribution even, but mycelium slightly thicker at the periphery than at the centre.

Type i. Pl. VI, Fig. 2. Aerial mycelium moderately plentiful and loose in texture. The distribution of the mycelium varied from one culture to another, in some it was evenly disposed, whereas in others there were radial and annular thicker portions.

Type j. Pl. VI, Fig. 5. Aerial mycelium plentiful, rather dense and evenly distributed.

Type k. Pl. VI, Fig. 5. Aerial mycelium fairly thin with strong radial markings at the centre of the colony.

Type l. Pl. VI, Fig. 5. Moderately plentiful aerial mycelium evenly distributed, except at the periphery where it is more plentiful.

Type m. Mycelium very thin, with practically no aerial mycelium. Distribution even.

Type n. Pl. VI, Fig. 3. Aerial mycelium moderately plentiful, loose and somewhat granular in appearance. Some variation in different colonies.

Type o. Pl. VI, Figs. 6 and 7. Aerial mycelium moderately plentiful at the periphery, but thinner at the centre. Considerable variation.

Type p. Pl. VI, Figs. 6 and 7. Aerial mycelium thin, generally thicker at the edge than at the centre. Some variation.

Type q. Pl. VI, Fig. 2. Aerial mycelium thin and loose, distribution even.

Type r. Pl. VI, Figs. 5 and 6. Aerial mycelium very thin with a narrow thicker band at the periphery.

Type s. Pl. VI, Fig. 2. Very thin aerial mycelium, evenly distributed and close to the surface of the medium. Inoculum always covered with a dense white mycelium.

Type t. Pl. VI, Fig. 3. Plentiful aerial mycelium, rather dense with radial markings. Variable.

Type u. Pl. VI, Fig. 4. Moderately thick aerial mycelium at the centre of the colony tapering off towards the periphery. Dense.

Type v. Pl. VI, Fig. 4. Very plentiful aerial mycelium, rather denser than types d and e. Mycelium unevenly distributed revealing a 'flecked' appearance by transmitted light.

Type w. Pl. VI, Fig. 4. Aerial mycelium plentiful. Some yellow coloration of the mycelium, generally more marked at the centre.

Type x. Pl. VI, Fig. 4. Aerial mycelium generally moderately plentiful, but subject to some variation. In all cases there is a narrow border of more plentiful mycelium at the periphery.

Type y. Aerial mycelium thin with some variation in radial markings.

Type z. Aerial mycelium moderately plentiful, loose, distribution uneven.

Type α . Pl. VI, Fig. 6. Aerial mycelium plentiful, loose in texture but with a denser zone at the centre.

Type β . Aerial mycelium plentiful, dense in texture, evenly distributed. Water-drops plentiful on the surface of the colony.

6. DISCUSSION.

A comparison of the segregants of any cross with their parents in the photographs in Pl. VI will show that (with the exception of type e which is indistinguishable from 30, and of several doubtful cases in which a segregant and its parent are very similar but not quite identical), in no case is it possible to pick out a particular type and say that it is identical with one or other of its parents. An examination of the growth-rates of the segregants and their parents in Text-figs. 8–14 also shows that only rarely does the frequency distribution of a segregant agree exactly with that of one of its parents, and even in such a case the two are frequently dissimilar in appearance. Since the segregants belong to the haploid generation the relative frequencies of occurrence of the different types (assuming the absence of linkage), should be equal, and where linkage is taken into consideration those segregant types having a greater number of factors in common with one or other parent should be in excess. Bearing this in mind, the only explanation which can be put forward to explain the rarity of types similar to their parents is that growth in pure culture over a

considerable period of time alters the appearance of a colony while its genotype remains unchanged. That growth in culture does alter the reactions of *Coprinus sphaerosporus* is evident from the decrease in fertility of diploid strains over a period of some months, though it has not been determined in these cases whether the genotype has altered or remained unchanged. A comparison of the growth-rate determinations of any one control strain made at different times and on different batches of media shows them to be very constant in most cases. An exception to this is found in strain 2 in which the growth-rate differed considerably from its original value (Text-fig. 11, 10 × 2) in subsequent determinations (Text-figs. 11, 13, and 14).

In considering the number of factors involved in any cross, it will be necessary to take into account the presence of the 'dwarf' type of segregant, i.e. those cultures in which the growth was limited so that the colony rarely became sufficiently large to be visible to the naked eye. Unfortunately it has not been possible, owing to the large numbers of single-spore cultures necessary, to determine accurately the frequency with which these dwarfs occur in any cross. Probably the most accurate estimate which has been made is that of 50 per cent. for the cross 15 × 16, though even in this case only two independent determinations were made. It is not at present possible therefore to estimate the number of factors concerned in the production of such dwarf colonies.

Since all the work on this fungus has been confined to the haploid generation it has not been possible to deal with the question of the dominance and recessiveness of the various factors concerned, nor, owing to the presence of several factor differences in each of the crosses made and to the interaction of the factors in most cases, has it been possible to determine which factors are allelomorphic to one another or how many factor differences are concerned in the various crosses.

A comparison of the frequency distributions of the segregants with those of their parents (and the controls) in the different graphs will show that in most cases the deviations in the distributions of the segregants are greater than those of the parents or controls which have comparable mean growth-rates. While a slightly greater deviation in the case of the segregating types would be expected owing to their being subcultured from different colonies, whereas all the representatives of each parental or control type were derived from the same culture, the increased deviations actually observed were much greater than would be expected from this cause alone. It therefore appears probable that some of the various segregating types are not homogeneous in the factors affecting their respective growth-rates. This is especially the case in types d and e (the only ones common to all the crosses) where the deviations are in all cases very large.

It is of interest to notice that in each of the crosses which have been made (with the exception of those in which strain 30 is concerned), the

rate of spread of certain of the F_1 haploids was greater than that of either parent. The d and e types, which as already stated were present as segregants from all crosses, were among these, and in each cross some of them had growth-rates of the order of 0.8 cm. per day. This rate of spread is comparable to that of strain 30 and is the most rapid which has been obtained in any haploid so far measured.

In the two experiments on inheritance in reciprocal crosses it was found that the same types were obtained irrespective of which diploidized parent was used to provide spores. Thus the same types were obtained when 21×10 was the diploid employed as when 10×21 was used. Text-figs. 10 and 12 show the frequency distributions of the segregants from the reciprocal crosses 10×21 and 21×10 and from 30×21 and 21×30 respectively. In each graph it can be seen that the distributions of similar types in reciprocal crosses were approximately the same in most cases, agreement being better where the frequencies of the type concerned were large. It is suggested that, so far as can be deduced from these results, there is no reason to suspect any difference in the segregants or their frequencies in reciprocal crosses.

A comparison of the segregants from the three diploids 21×10 , $21/10 \times 2$, and 10×2 (*vide* Table I) shows that apart from strains d, e, and h, which were common to all three crosses, and of f, u, y, and β which were peculiar to 10×2 , all the segregant types were common to $21/10 \times 2$ and either 21×10 or 10×2 . No type therefore was found in $21/10 \times 2$ which was not also found in one or other (or both) of the two remaining crosses. Of the six types common to $21/10 \times 2$ and only one of the other crosses, three, namely, g, n, and t, were found in 21×10 and three, namely, v, w, and x, in 10×2 . A similar result was obtained in the three crosses 21×30 , $21/30 \times 2$, and 30×2 , namely, that no type (with the exception of α which occurred with the small frequency of 1 per cent.) was found in $21/30 \times 2$ which was not also found in either 21×30 or 30×2 , and that three strains f, o, and p, were peculiar to $21/30 \times 2$ and 30×2 and strain r to $21/30 \times 2$ and 21×30 . Types d and e were common to all three strains, but whereas in the first three crosses they occurred with a frequency of about 40 per cent. in each cross, in this instance they had the following frequencies in each of the three crosses: 21×30 , 90 %, $21/30 \times 2$, 96 %, and 30×2 , 59 %. It is thus seen that the frequency with which d and e occurred in the cross $21/30 \times 2$ is much nearer to the frequency found in 21×30 than to that found in 30×2 . From the foregoing results it is deduced that in a diploid such as $21/10 \times 2$ or $21/30 \times 2$, three different types of nuclei are to be found, and that on the production of basidia by such a diploid the three nuclear types fuse with one another in pairs in all possible ways (having regard to their different sexes) and that each type of fusion nucleus gives rise to basidiospores in the ordinary way. Thus in strain $21/10 \times 2$, a nucleus (21)

will pair and fuse with a nucleus (10), and (10) will pair and fuse with (2), and in each case basidiospores will be formed following the reduction division of the fusion nucleus. This conclusion is also supported by the results obtained from the diploid $30/21/10 \times 2$ in which four different nuclear pairs are possible, namely, (10) and (2), (21) and (10), (30) and (21), and (30) and (2). Of the segregants from this cross types d and e are common to all crosses, type f is found in the segregants of 10×2 and 30×2 but not in those of 21×10 or 30×21 . Types g and n are peculiar to 21×10 , and p is confined to the cross 30×2 . Also the frequency of types d and e is closer to that found in the cross 30×21 than to that found in any of the other three crosses. From this it seems probable that segregants from all four nuclear pairs may be represented in the segregants of $30/21/10 \times 2$. (Type z which is peculiar to this diploid occurs with the small frequency of only 2 per cent., so that its absence from the segregants of any of the other crosses is not surprising in view of the small numbers available for examination in any one case.)

A comparison of the frequencies with which the different types occur in the segregants from either 'compound' diploid, i.e. $21/10 \times 2$ or $21/30 \times 2$, with the frequencies of similar types in the two simple related diploids is subject to considerable difficulty arising out of the want of reliance which can be placed on the small frequencies of certain types. In segregants from the diploid $21/10 \times 2$, five types, namely, h, t, v, w, and x, have frequencies either intermediate between those of the respective types from the crosses 21×10 and 10×2 , or else respectively smaller than those of the corresponding types from one or other of these strains, where they are not represented in both. In the three types d and e, g, and n the frequencies are respectively slightly greater than with either (or one or other) of the two simple diploids. Similarly in $21/30 \times 2$ types f, o, p, and r, occur respectively with smaller frequencies than they do in the corresponding types from one or other of the simple diploids 21×30 and 30×2 . Type d and e, on the other hand, has a greater frequency in the segregants from the compound diploid than among those from either of the simple ones. These results, taken as a whole, are what would be expected if $21/10 \times 2$ and $21/30 \times 2$ were (from the nuclear point of view) the equivalent of mixtures of the simple diploids 21×10 and 10×2 and of 21×30 and 30×2 respectively. As regards which of the nuclear pairs plays the greater part in the production of basidiospores in either $21/10 \times 2$ or $21/30 \times 2$, the relative frequencies of all the types taken together, and especially of the types g, h, and n in the former case and of d and e in the latter, point to the nuclear pairs (21) and (10), and (21) and (30) respectively as the more important in each strain. In the case of $30/21/10 \times 2$, the frequency of type d and e is 87 per cent., and in the crosses 30×21 , 30×2 , 21×10 , and 10×2 , 90, 59, 43, and 40 per cent. respectively. Thus the three compound diploids agree

in that, owing to the manner in which the diploid was built up, it is the last type of nuclear pair which may be formed which is the most important as regards spore production. The strains $21/10 \times 2$ and $21/30 \times 2$ have the haploids 21 and 2 in common. These particular compound strains were used (when others composed of altogether different haploids might have given more valuable results), owing to the difficulty in obtaining three strains of appropriate sex which differed in growth-rate and appearance, and which would give fertile diploids when paired in the manner required. Out of many strains tested, 30, 21, 10, and 2 were the only ones which fulfilled the necessary conditions.

From the above it would appear very probable that the diploids $21/10 \times 2$ and $21/30 \times 2$ each contains three different nuclei which are in a position to form two pairs (21, 10) and (10, 2) in the first and (21, 30) and (30, 2) in the second case, and that these pairs can fuse together and give rise independently to basidiospores.

Similarly in the diploid $30/21/10 \times 2$ there are grounds for believing that the four possible nuclear pairs (30, 21), (30, 2), (21, 10), and (10, 2) may all be present (or potentially present) in the mycelium, and that each pair can fuse and the fusion nucleus give rise to four spores in which the characters of the parental nuclei segregate out. It also appears that in the diploid $21/10 \times 2$ the nuclear pair (21, 10), in $21/30 \times 2$ the pair (21, 30), and in $30/21/10 \times 2$ the pair (30, 21) are the most common.

It has been suggested by Rawitscher (4), in an attempt to explain Buller's observations (1) on *C. lagopus*, that in illegitimate combinations such as $(AB) \times (Ab) + (aB)$ the haploid mycelium receives both the nuclei of the diploid $(Ab) + (aB)$, and that the latter divide conjugately and displace the original (AB) nuclei. In the present instance it has been possible to show that an analogous happening does in effect take place, and that the nuclei of the diploid both enter the haploid and proceed to divide. Beyond this the analogy breaks down as the nuclei of the haploid, which are assumed to take no further part in the development in *C. lagopus* owing to their 'illegitimacy', proceed, in *C. sphaerosporus*, to pair with the appropriate nuclei of the diploid and form a second set of dicaryons. The exact origin of those nuclei of the diploid which pair with the nuclei of the haploid is not known. Either they are withdrawn from membership of the dicaryons (established in the original diploid) under the influence of the nuclei of the haploid, or else in the formation of the original diploid some nuclei remain unpaired, and are thus free to enter the haploid mycelium unattached and to pair with the nuclei of that mycelium. However these new dicaryons may be formed, they are more numerous than are dicaryons of the original diploid. It might be possible to throw some light on the origin of the nuclei of the original diploid which pair with those of the haploid mycelium by employing the reciprocal diploid strains

and comparing the segregants. Thus, if strain A is paired with B, in the resulting $A \times B$ diploid there will be present (A, B) dicaryons, and if any free nuclei are also present they are more likely to be of the (A) than (B) type, whereas in the diploid $B \times A$ free nuclei are more probably (B) than (A), (or at least the proportion of (B) nuclei is likely to be greater than that of (A) nuclei). If, then, a mycelium C of opposite sex to A is taken and paired with $A \times B$ and again with $B \times A$, the diploids $C/A \times B$ and $C/B \times A$ will be obtained and their segregants can be compared. Should it be found that in the former case there is a preponderance of spores derived from (C, A) nuclei (as is the case in the experiments described above), whereas in the latter (A, B) derivatives predominate, it would appear very probable that the nuclei (C) are unable or are only rarely in a position to separate (A) nuclei from a dicaryon and that the large number of (A, C) segregants obtained from the $C/A \times B$ cross were derived from the union of (C) and unattached (A) nuclei which had not formed dicaryons when the mycelium A had been diploidized by B in the first instance. In order that a haploid nucleus may be able to split up a dicaryon, it must have a greater attraction for that nucleus of opposite sex to itself than has the other member of the dicaryon. In the present instance this would imply that in the mycelia $21/10 \times 2$, $30/21/10 \times 2$, and $21/30 \times 2$ respectively, (21) had a greater attraction for (10) than had (2), (30) had a greater attraction for (21) than had (10), and (21) a greater attraction for (30) than had (2), and it is unlikely that this should be so when it is remembered that the various members of these compound mycelia were selected at random. Professor A. H. R. Buller has suggested to the author another interpretation of how the (C, A) dicaryons may be formed. This is that under the influence of a (C) nucleus the (A) nucleus of an (A, B) dicaryon may be stimulated to divide and one of its daughter nuclei form a dicaryon with the (C) nucleus while the other is attached to the (B) nucleus. The objection to the previous hypothesis would not apply in this case, as it would no longer be necessary to assume in each instance a greater attraction between the nucleus of the haploid and that nucleus of the dicaryon of opposite sex, than exists between the two nuclei of the dicaryon. The experiment suggested above, in which reciprocal diploid strains are severally paired with a haploid and the segregants of the two resulting diploids compared, may also serve to distinguish between Buller's hypothesis and the suggestion, put forward above, that the (A) nuclei enter the C mycelium unattached and are consequently free from the first to form dicaryons with the (C) nuclei.

7. SUMMARY.

A method for the measurement of the growth-rate of fungi in test-tube cultures has been devised, and an apparatus designed to provide

increased accuracy in the determination of the diameter of a fungal colony.

The segregants from various diploid mycelia have been grown and their growth-rates determined. They have been separated on morphological grounds into twenty-eight different types. Some 8,000 cultures have been employed in these determinations, and growth-rates have been measured in about 3,500 colonies.

Factors have been shown to be present in most crosses which limit the growth of the affected colony to a diameter of less than a millimetre. It was not possible to determine the percentage of such colonies among the segregants of any one cross, but it probably ranges between 15 and 50 per cent. for the particular crosses examined.

It was found that in reciprocal crosses similar types of segregants were produced, and that each type had approximately the same frequency of occurrence.

Owing to numerous factors segregating out in each cross and to their interaction in the relatively undifferentiated mycelium, it has not been possible to estimate the number of factors involved or to say which factors are the allelomorphs of one another.

By comparing the segregating types and their frequencies produced by a compound diploid such as $C/A \times B$ (in which the haploid C was diploidized by the diploid $A \times B$) with the two simple related diploids, $C \times A$ and $A \times B$, it has been found possible to show that in the compound diploid two groups of dicaryons are probably present, namely (C, A) and (A, B) and that both take part in the production of basidiospores when the mycelium gives rise to fruit-bodies. It has also been shown that the (C, A) nuclei are relatively more plentiful than the (A, B) nuclei.

It is suggested that in the formation of the mycelium $C/A \times B$, (A, B) dicaryons enter the C mycelium and the (C, A) dicaryons probably arise from the union of (C) nuclei with (A) nuclei, which have remained unattached to the (B) nuclei of the $A \times B$ mycelium and have subsequently entered the C mycelium. It is considered that the alternative suggestion, namely, that (A) nuclei are separated from their union with (B) when subjected to the influence of the nuclei of the C mycelium and subsequently form (A, C) dicaryons with the latter, is improbable, and occurs but rarely, if at all.

I have much pleasure in thanking Professor F. W. Oliver for the advice and encouragement he has given while these experiments were in progress.

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EXPLANATION OF PLATE VI.

Illustrating Dr. Hugh Dickson's paper on 'Studies in *Coprinus sphaerosporus*'.

All the photographs were taken by reflected light, and the colonies were grown on malt-agar in test-tubes.

Figs. 1-7. Each of the seven figures represents typical examples of the segregating types obtained from a particular cross. To the left of each photograph (with the exception of fig. 4, which shows the segregants of the cross 10×2), the parental strains are represented. The reference letters and figures of the segregants and their parents respectively are given above each culture.

Fig. 1

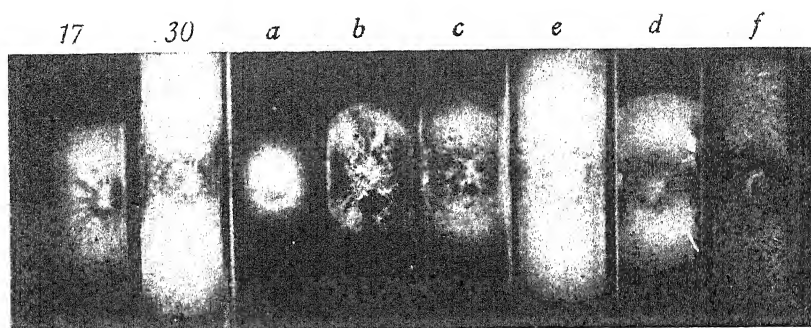


Fig. 3

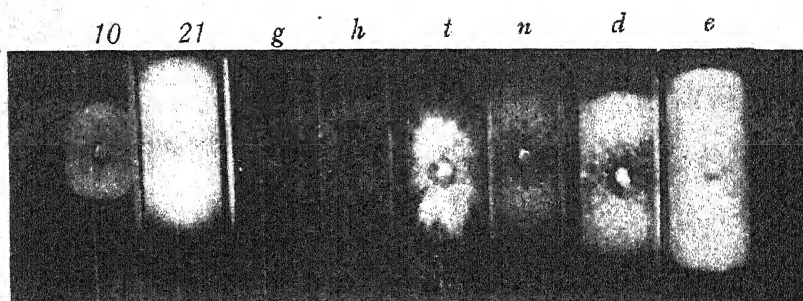


Fig. 5

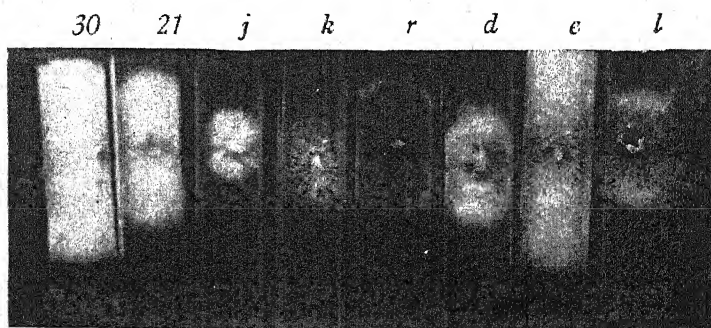


Fig. 2

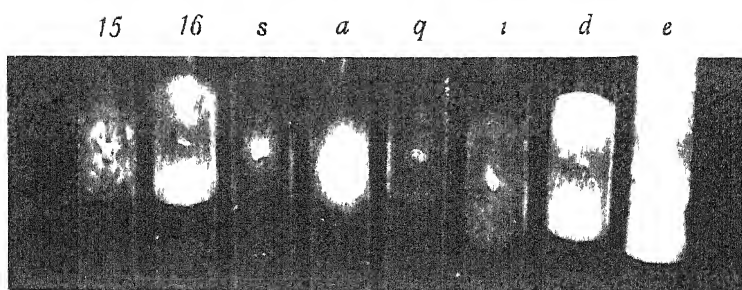


Fig. 4

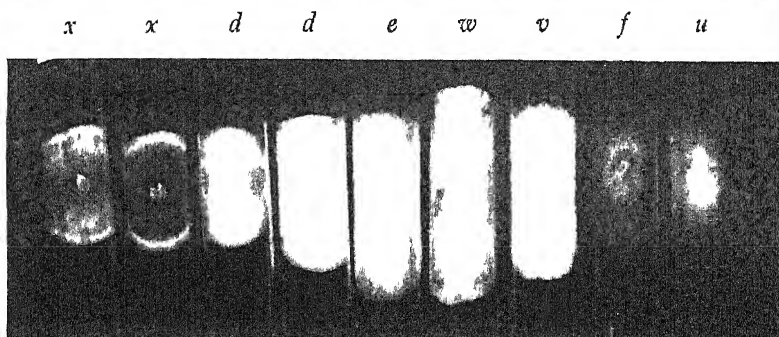


Fig. 6

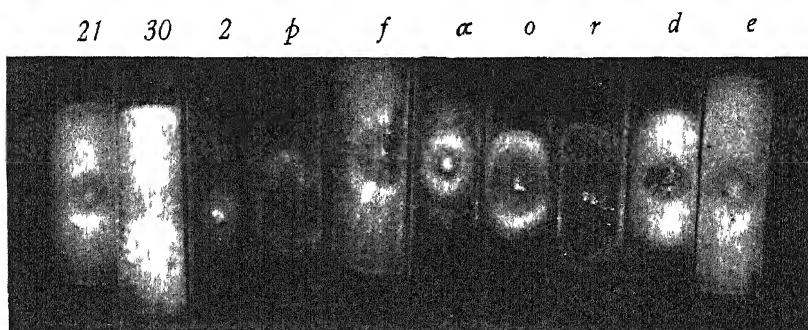
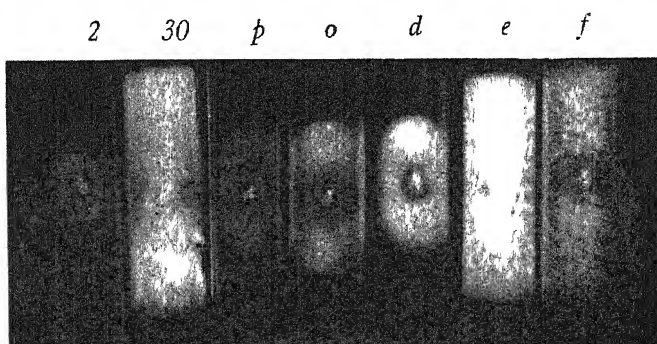


Fig. 7



Two New Members of the Chaetophorales from Egypt.

BY

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With Plate VII and five Figures in the Text.

1. OLIVERIA TERRESTRIS N. GEN. ET SP.

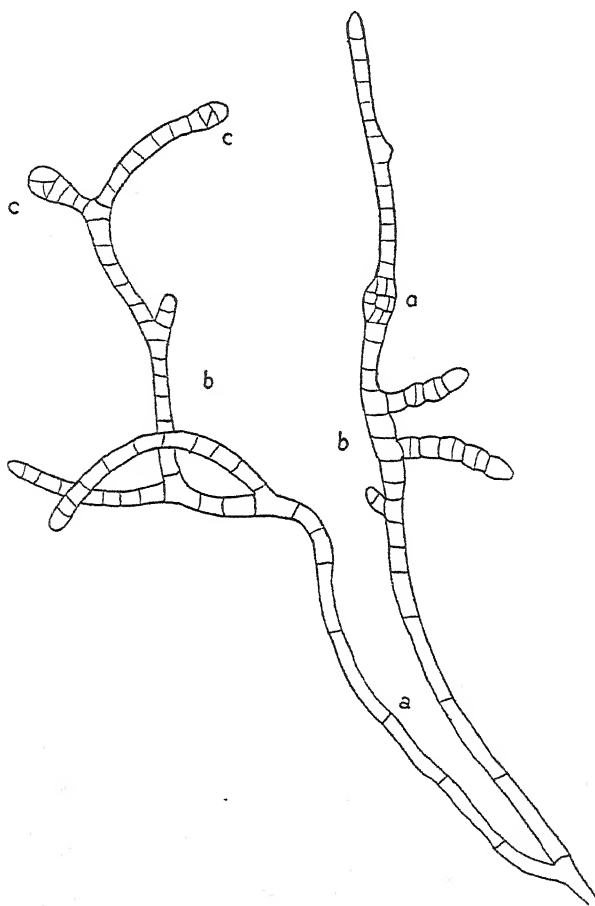
THIS alga was growing together with *Protosiphon* and *Botrydium* on bare cultivated land near the Timsah Lake, Ismailia, in March. It forms brown-coloured patches on the surface of the dry soil, but on the shady sides of the cracks in the mud where the soil is damp the growth is green. The mud is rich in clay and sticky, so that it was found necessary to study the habit and method of growth of the alga by cultivating it on agar media. Pieces of the original filaments were transferred to sterilized cultures composed of agar with Knop's or Bristol's media and placed in Petri dishes. After about three weeks green patches like those found in nature were formed in the agar. Some of the dishes were allowed to dry. As the agar consolidated and shrank away from the walls of the dishes the green patches assumed a brown colour. Subsequent moistening of the agar with sterilized distilled water led to a reassumption of growth, new green zones appearing at the margins of the brown ones (Plate VII, Fig. A).

The plant body is composed of two types of filaments:

1. *Long-celled filaments* (Text-fig. 1, a) which penetrate vertically into the agar to a depth of 1–2 mm. and then grow more or less horizontally through it. These filaments are uniseriate and branch extensively. The branches originate singly as lateral outgrowths from the upper end of the parent cell just beneath the septum. The cells of these filaments are thin-walled, elongate, and comparatively narrow (four to five times as long as broad). Each cell has a single, curved, parietal, plate-shaped chloroplast, which lies against part of the longitudinal wall and extends the whole length of the cell. The chloroplast contains several pyrenoids (three to five). There is a single central nucleus.

2. *Short-celled filaments*. The long-celled filaments may themselves

curve upwards or give rise to lateral branches which grow vertically and appear above the surface of the substratum (Plate VII, Fig. B). After some time the apical growth ceases and the cells divide transversely to form the short-celled filaments (Text-fig. 1, *b*). Generally the divisions become

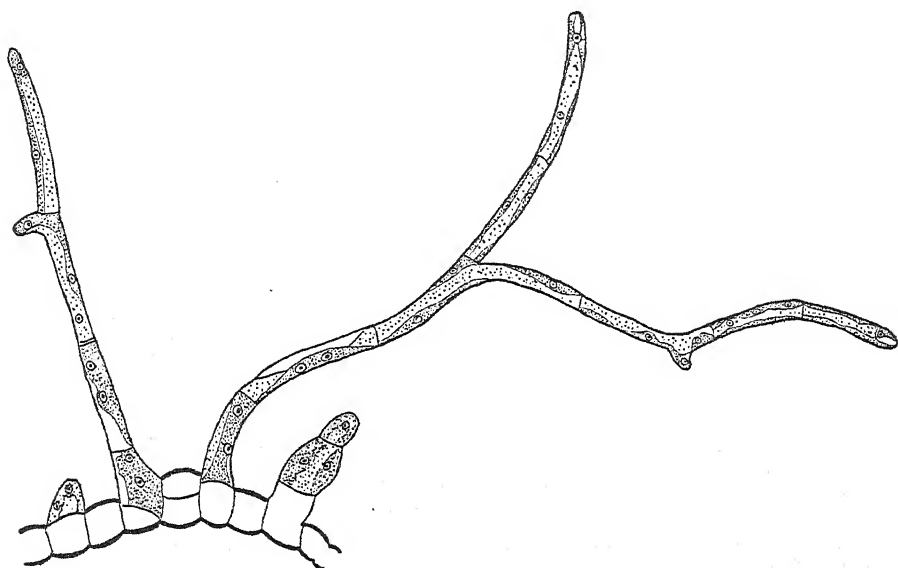


TEXT-FIG. 1. *Oliveria terrestris* sp. nov. *a*, Long-celled filaments, giving rise to short-celled filaments, *b*; *c*, club-shaped terminals; *d*, swollen portion due to localized division of some of the cells. $\times 450$.

irregular, leading to the production of multiserial filaments or of cell-groups (Text-fig. 4). The short-celled filaments branch extensively in the same way as before. The terminal part of the filament may be club-shaped (Text-fig. 1, *c*) and (Text-fig. 4), and the end cell may or may not divide in different directions. The short-celled filaments may show swollen portions due to localized growth, and division taking place in some of the cells (Text-fig. 1, *d*). The cells of the short-celled filaments have dense contents, a single

nucleus and a single curved plate-shaped parietal chloroplast with one to three pyrenoids.

When exposed to drought the short-celled filaments, and especially those above the substratum, acquire thick walls and brown-coloured



TEXT-FIG. 2. *O. terrestris* sp. nov. Thick-walled cells (akinetes) producing long-celled filaments in agar culture. $\times 520$.

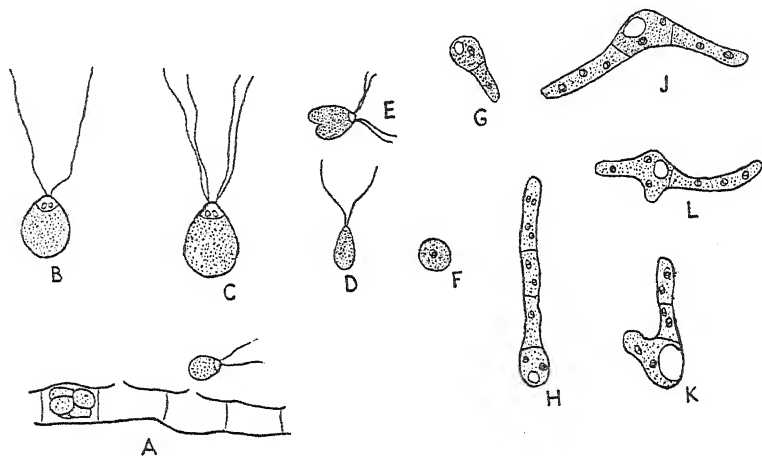
contents (Text-fig. 4), while at the same time the contents of the long-celled filaments decrease in amount and gradually disintegrate and ultimately the cells die.

The brown-coloured thick-walled cells are most probably akinetes which form the perennating stage of the alga. They probably dissociate and are likely to be disseminated by water or wind. They can withstand drought.

Vegetative reproduction. The cells of the short-celled filaments, whether green or brown, are capable in the presence of moisture of growing freely. The outer thick walls, when present, dissolve and the cells elongate to form long-celled threads (Text-fig. 2), which creep over the surface of the agar and sooner or later penetrate into it.

Asexual reproduction. The cells of the short-celled filaments produce zoospores when submerged in water for one to three days. The contents divide up into four to eight units, which round off and escape through a lateral aperture (Text-fig. 3, A). The swimmers are more or less rounded and either biciliate (Text-fig. 3, B) or quadriciliate (Text-fig. 3, C), the cilia being about twice the length of the swimmer. In the colourless anterior

end lie two contractile vacuoles. There is a single parietal chloroplast and a central nucleus. There is no eye-spot, but zoospores produced from cells with brown contents sometimes show brown pigment.



TEXT-FIG. 3. *O. terrestris* sp. nov. A, formation and escape of swarmer; B, Biciliate zoospore; C, Quadriciliate zoospore; D, gamete; E, fusion; F, resting spore; G-L, various stages in the germination of zoospores. A $\times 375$; B and C $\times 900$; D and E $\times 750$; F-L $\times 420$.

After swarming, the zoospore comes to rest, rounds off, surrounds itself with a wall (Text-fig. 3, F) and germinates directly. One or more filaments arise from the germinating spore, and grow in different directions (Text-fig. 3, G-L). The filaments creep over the surface of the substratum for some time and then penetrate into it.

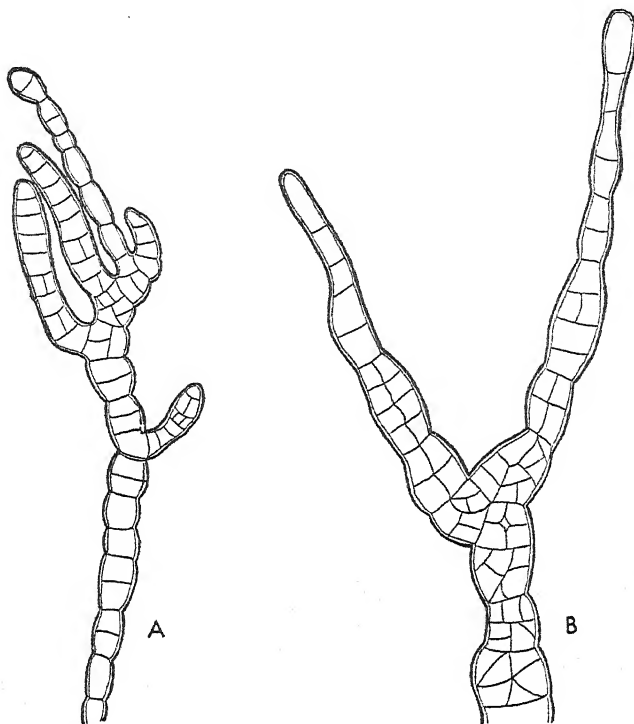
Sexual reproduction. Under certain conditions isogamous gametes are produced, which are pear-shaped and biciliate (Text-fig. 3, D). They show the same structure as the zoospores, but are smaller and produced in larger numbers from the parent cell. The fusing gametes become entangled by their cilia, continue to move jointly for a short time and then fuse laterally (Text-fig. 3, E).

The zygote rounds off, surrounds itself with a membrane and germinates in the same way as the zoospore.

Systematic Position.

This alga may be referred to the Chaetophoraceae in view of the mode of branching and the absence of any specialized reproductive organs. Diverse terrestrial forms have already been recorded in that group, and it is not unlikely that more will be discovered. The habit of the Egyptian alga is like that of Dangeard's *Rhizothallus* (2), but the latter has a complex rhizoidal system which is lacking in the present form. The cell groups and perennating cells in the short-celled filaments recall those of Iyengar's

Frittschiella (3), but it again differs from Frittschiella in the absence of specialized structures like rhizoidal, prostrate and projecting systems. The presence of a pigment (probably haematochrome) in the cells, which is



TEXT-FIG. 4. *O. terrestris* sp. nov. Aerial brown-coloured filaments with thick walls showing branching, cell groups, and club-shaped ends. $\times 375$.

characteristic of many terrestrial members of the Trentepohliaceae is probably merely a terrestrial adaptation and does not signify any relationship. In general habit and structure this alga does not show any close affinity with any form described, and I am therefore making it the type of a new genus of Chaetophoraceae for which I suggest the name.

Oliveria gen. nov.

Terrestrial; thallus differentiated into long-celled and short-celled filaments, with apical and diffuse growth respectively; filaments extensively branching, terminal cell not attenuated, no hairs or setae; aerial cells with brown pigment; chloroplast single parietal with a number of pyrenoids; zoospores bi- or quadri-ciliate; gametes isogamous, biciliate, the zygote germinating direct; akinetes with thick walls and brown contents formed from short-celled threads.

Oliveria terrestris sp. nov.

Characters as for genus; long-celled filaments $4-7\ \mu$ broad, cells three to five times as long as broad; short-celled branches $14-17\ \mu$ broad, cells nearly as long as broad; gametes $(3.5-4\ \mu) \times (7.5-10\ \mu)$; zoospores; biciliate $(8-10\ \mu) \times (9-12\ \mu)$, quadriciliate little larger, cilia about twice the length of the swimmers.

2. PSEUDOLEPTOSIRA CALCAREA N. GEN. ET SP.

This alga was found in Egypt growing epiphytically on aquatic plants in two different localities, namely: on *Cladophora* in a main drain at 'Elwadi' in Fayum Province at the beginning of February, and in a permanent pool at Giza on the stems of *Zannichellia palustris* about the end of the same month.

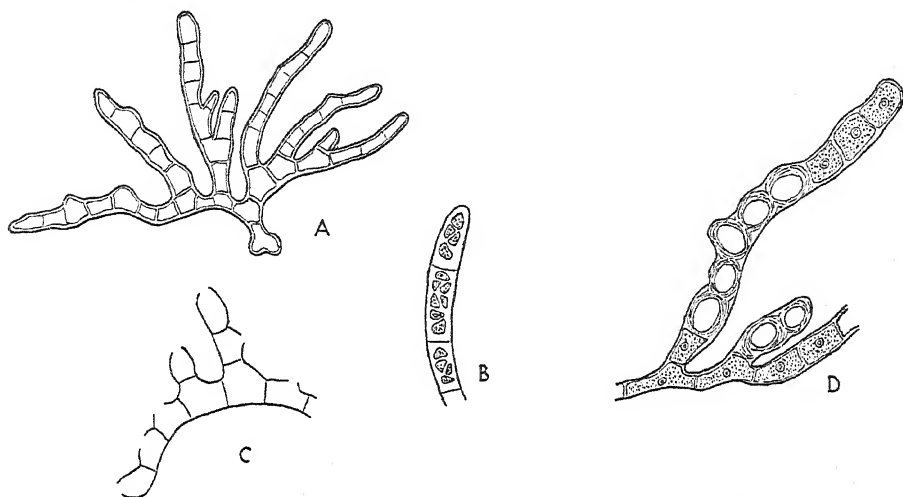
To the naked eye, the alga appears as small dark green, cushion-like masses scattered on its host. The dark colour is due to dense incrustation with carbonate of lime, which forms a complete covering to the alga except for the tips of the filaments which are free. To study the form and structure of the plant the material has to be treated with dilute acid to dissolve the lime incrustation.

The alga is firmly attached to the substratum by means of a lobed basal cell, which bears two, sometimes more, main filaments that diverge in various directions. The main filaments grow more or less parallel to the surface of the substratum and give rise unilaterally, on the side away from the substratum, to branches, which stand off and grow on the upward direction (Text-fig. 5, A). These laterals may branch once or twice again, so that a crowded group of projecting filaments results. The branches arise singly from the upper end of the parent cell just beneath the septum. The apices of the branches are rounded and no hairs are formed. The cells are generally as long, to twice as long as broad, but the terminal cells, especially in young plants, are more elongated (two to three times as long as broad). The cells are uninucleate. The chloroplast is single and parietal, covering all faces of the wall and containing one (rarely more) central pyrenoid. The cell wall consists of two layers, the innermost is thick, homogeneous, and is composed of cellulose. The external layer is very thin and consists of pectin.

In mature plants the contents of many cells were found dividing into four to eight, sometimes more, portions (Text-fig. 5, B). Some other cells were found to be empty, with an aperture in the wall (Text-fig. 5, C), a formation of motile reproductive cells is suggested, although none such were actually seen. In the apical cells the aperture in the wall is terminal, whilst in the others it is lateral. The cells producing these reproductive units do not differ in any other way from the vegetative cells.

Rounded or oval cells with thick lamellated walls and dense contents were commonly seen (Text-fig. 5, D). They are probably akinetes.

In habit the alga is very similar to *Leptosira* (1), but it differs markedly from it in the absence of a creeping system, and in the fact



TEXT-FIG. 5. *Pseudoleptosira calcarea* sp. nov. A, complete specimen showing habit and rhizoidal cell; B, cells dividing into a number of reproductive units (Zoospores?); C, empty cells with apertures through which reproductive units escaped; D, a small branch with akinetes; other cells showing structure, each with a single pyrenoid. A and C $\times 270$; B and D $\times 375$.

that attachment to the substratum is effected by a single basal cell. In the latter respect it resembles *Microthamnion* (4), and differs from most other Chaetophorales. Another difference from *Leptosira* is seen in the presence of pyrenoids in the chloroplast. It should clearly be referred to the Trentepohliaceae of Fritsch (5), and, in view of the single chloroplast and absence of haematochrome, is best included in the *Gongrosireae* (5).

Pseudoleptosira nov. gen.

Thallus aquatic, epiphytic; attached to the substratum by a single rhizoidal cell, the latter bearing a number of unilaterally branched filaments consisting of a dense cushion-like mass; encrusted with carbonate of lime; filaments not tapering and no hairs; chloroplast single and parietal, covering all faces of the wall with one pyrenoid, sometimes more; reproduction by akinetes and zoospores (?); Sporangia not swollen.

Pseudoleptosira calcarea n. sp.

Characters as for genus; filaments 10–14 μ in diameter; cells one to two times as long as broad except the terminal cells which are one to three times as long as broad; akinetes 8–10 μ in diameter.

The author wishes to express his gratitude to Professor F. E. Fritsch for his guidance and suggestions throughout this work, and to Professor F. W. Oliver for his unfailing assistance. His thanks are also due to Miss F. Rich, Dr. N. Carter, and Y. S. Sabet for useful suggestions.

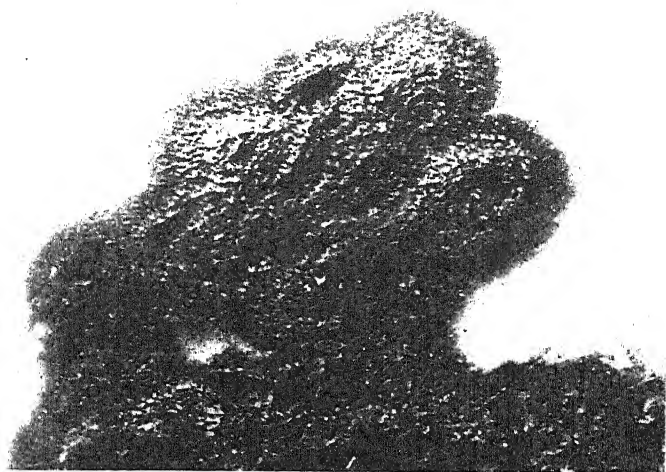
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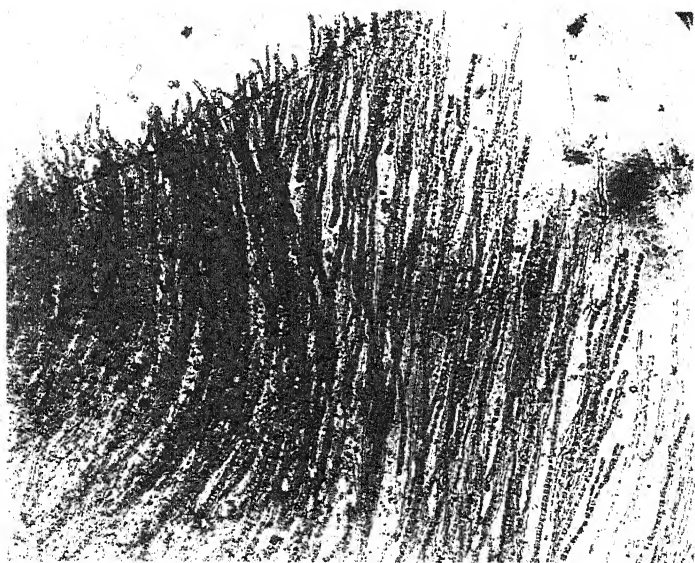
EXPLANATION OF PLATE VII.

Illustrating Mr. A. A. Nayal's paper on 'Two New Members of the Chaetophorales from Egypt'.

Olivuria terrestris sp. nov. Fig. A, surface view of an old agar culture showing brown-coloured zones of growth. Fig. B, vertical section of agar culture showing short-celled filaments curving upwards towards the surface of the agar ; some filaments have already appeared over the surface. A, \times about 2.5 ; B, \times about 150.



A



B

NAYAL - CHAETOPHORALES.

Transpiration and Pressure Deficit.

I. Apparatus and Preliminary Experiments.¹

BY

F. M. HAINES, PH.D.

With three Figures in the Text.

INTRODUCTORY AND THEORETICAL.

IT appears to be generally accepted that leaf water content is one of the principal factors controlling transpiration rate. The leaf water content depends, however, in turn partly upon the conditions of pressure or tension in the conducting tracts supplying the water. The transpiration rate might, therefore, be expected to bear some relation to the tract pressure, and the leaf water content would appear to be only a sort of passive intermediary through which the transpiration rate might be affected indirectly by changes in the pressure or tension in the tracts. The actual tract pressure, however, will not be the operating factor, but the difference between the tract pressure and that of the atmosphere, already termed by the writer the pressure deficit² (3, p. 680).

In view of these considerations it appeared desirable to attempt to investigate the relation between transpiration rate and the pressure in the conducting tracts, or rather the pressure deficit, by direct experiments. Such work has also been shown to be desirable from the point of view of evaluating 'drought resistivity' (cf. Haines (3), p. 683, and p. 683 footnote).

Experiments with this object may be performed in two ways:

(a) The transpiring surfaces of a cut leafy branch may be freely

¹ From the Botanical Department, Queen Mary College, London.

² The term 'negative pressure' to denote reduced pressure in the tracts is avoided, as it leads to confusion through classifying all pressures (or tensions) below atmospheric pressure as 'negative' instead of only those below zero pressure (i.e. true tensions). The term 'pressure deficit' is therefore used instead and is defined as the difference between the pressure in the tracts and the pressure in the atmosphere, tensions in the tracts reckoned as negative pressures in the true sense, i.e. relative to zero pressure. Thus when the actual pressure in the tracts is zero, the pressure deficit is 1 atm. If there be a tension in the tracts of 9 atm. the pressure deficit is 10 atm., and so on. Cf. Haines (3), pp. 679-80.

exposed to the atmosphere, but the branch allowed to take up water from a closed vessel in which the pressure is either naturally reduced (by the absorption) or artificially (e.g. by a pump); or

(*b*) the difference between the pressure in the tracts and the pressure of the atmosphere may be secured by allowing the plant to take up water from an open vessel at atmospheric pressure, the transpiring parts of the plant, however, being enclosed in a strong metal cylinder in which the pressure is increased above that of the atmosphere.

By method (*a*) only the effects on transpiration rate of pressure differences (pressure deficits) less than one atmosphere can be investigated, but by method (*b*) the pressure may be made anything up to 30 atmospheres or more, according to the nature of the plant used. In method (*b*) the same pressure *differences* (pressure deficits) may be obtained as in nature, but the actual values are all moved up the scale. In both methods, however, the results of the pressure deficits are in all respects analogous to those of similar deficits in nature so far as the effects upon transpiration rate and also upon the 'leaf-pull', or force causing flow from the stem to the leaves, are concerned.

In a third method of experimentation the plant may be allowed to take up water from a special form of vessel which can be closed as in method (*a*), or opened at will, and the rates of absorption and transpiration and the leaf water content may be measured simultaneously and independently at different deficits.

The relation of transpiration rate to pressure deficit has now been investigated by all these different methods, but inasmuch as method (*b*) is the only one capable of giving results over a wide range of pressure deficits, and as all the broader facts have been arrived at by this method, only the experiments of this type will be dealt with in this communication. Some interesting facts have also been obtained by the other methods, but these will be dealt with separately in a later paper.

Theoretical Considerations in Connexion with Method (b).

In method (*b*), the pressure cylinder method, the pressure in the cylinder deciding the pressure deficit is controlled, and the readings taken are those of the rate of absorption by the cut end of the branch. It is important to realize, however, that these rates of absorption which are measured are in all cases only taken as indicative of the water relations between the stem and the leaves. They have *no relation whatever* to the changes which would have been induced in the rate of absorption (by the roots) of the intact plant, or even to the rate at which water would pass from the roots to the stem of the intact plant under similar deficits. They will, in fact, be almost invariably opposite in sense to these absorp-

tion rates under natural conditions in consequence of the fact that in nature when a pressure deficit occurs the source of supply comes to be under higher pressure than the contents of the tracts (and the true absorption rate is increased), whereas in these experiments the pressures of the contents of the tracts and of the source of supply are made equal throughout by exposing the cut base of the stem to a supply at atmospheric pressure. The pressure gradient in the tracts themselves must be relatively very small and is here ignored. In nature the supply is kept at the same pressure as that of the air surrounding the evaporating surfaces, whereas in these experiments the supply is kept at the same pressure as the tract contents. Whereas in nature, therefore, an increase in the deficit will tend to increase the rate of absorption by an intact plant through its roots, in the experiments an increase in the deficit will have no *direct* effect upon absorption by the base of the cut stem (the tracts and supply being under equal pressures throughout), but will affect absorption by the cut stem only in so far as it reduces (or opposes) the tendency for flow to take place from the stem to the leaves: that is in proportion to the extent to which it alters the foliar forces lifting water. Throughout this paper, then, the term 'absorption' always implies only absorption by the cut base of the branch as in the experiments. The effects on ordinary absorption would definitely not be parallel to the effects on 'absorption' in this sense. 'Absorption' rates measured are indicative only of the leaf-pulls developed or of the rates of flow which would tend to take place in natural circumstances from stem to leaf under the influence of the deficits in question. The variations in this flow and in the absorption rates observed may be due partly to different transpiration rates and partly to changes in the volumes of the leaf-cells. This point, however, will be fully discussed under the experiments of Series VI in the next paper.

In the experiments to be described in this and the following paper (method (*b*), above), the plant or leafy branch, as already indicated, is enclosed in a cylinder, and the transpiring parts are exposed to increased pressure. The base of the stem passes out through the bottom of the cylinder through a special seal, and is allowed to take up water at atmospheric pressure.

In adopting this type of experiment, the line of argument was taken that the action of a reduced pressure or even of a tension in the tracts in nature upon such quantities as the transpiration rate, the leaf water content, &c., is entirely the result of the fact, not that the absolute pressure is low or absolute tension high, but that a difference in pressure exists between the contents of the tracts and the atmosphere. It is entirely the pressure deficit¹ or difference from the pressure to which the transpiring surfaces are exposed, which is operative, and not the absolute value of the pressure or tension in the tracts. Proof of this is given below, pp. 217-18.

¹ See footnote, p. 213.

This being so, the rate of transpiration and the leaf water content should be the same whether, for instance, the leaves be exposed to atmospheric pressure and the contents of the tracts to a tension of nine

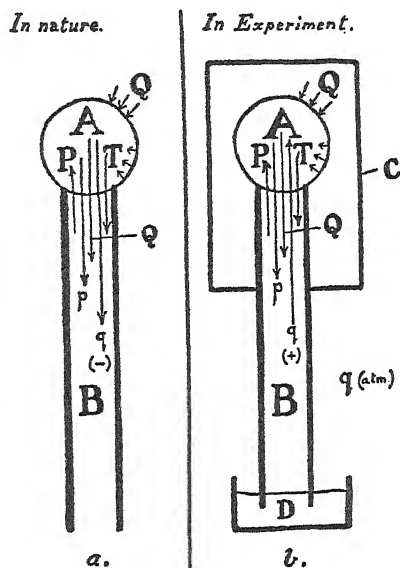


FIG. 1. Diagram to show the relations between the transpiring cells of the leaf and the conducting tracts of the stem; *a*, in natural conditions and *b*, during the conditions of pressure-cylinder experiments. A = transpiring leaf cell, B = conducting tract, c = pressure cylinder, D = artificial source of supply at atm. pressure. Q = pressure at leaf cell surface, *q* = hydrostatic pressure in conducting tract (negative during tensions in nature), P = osmotic pressure of leaf cell sap, *p* = osmotic pressure of contents of conducting tract, T = turgour pressure in leaf cell walls. The directions of the forces due to each factor are indicated by arrows. For further explanation see text, pp. 216 and 217.

atmospheres, or the leaves be exposed to a positive pressure of eleven atmospheres and the contents of the tracts to atmospheric pressure. The difference or 'pressure deficit' would be the same in both cases (10 atm.), and this is the value upon which the effects depend. The relations may be stated more completely by reference to Fig. 1.

If A represent a transpiring cell of the leaf, and B represent a conducting tract of the stem with which A is in connexion, the water relations of the stem and leaf may be taken quite simply as expressed by, or equivalent to, the relations between these two elements.

- Let *Q* = the actual pressure (in the cylinder) at the leaf-cell surface,
q = the actual hydrostatic pressure in the tract (tensions being reckoned as negative),
P = the osmotic pressure of the leaf-cell contents,
p = the osmotic pressure of the contents of the tract,
T = the turgour pressure of the leaf-cell.

Let it, moreover, be assumed that the contents of the leaf-cell be fully exposed to the pressure, Q , to which the leaves are exposed on account of the lack of rigidity of the leaf-cell walls; and that the contents of the tract, B , are not directly affected by or subjected to the pressure, Q , on account of the fact that the walls of the tract are rigid.

Then if a semipermeable protoplasmic membrane or a chain of intermediate living cells with protoplasmic membranes exist between the contents of A and the contents of B , the forces tending to cause movement of water from B to A will be due to the osmotic pressure of the leaf-cell contents and, in case b , the hydrostatic pressure in B .

Thus, Forces causing movement upwards = $P + q$.

The forces tending to cause movement of water from A to B will be due to the osmotic pressure of the tract contents, the turgour pressure of the leaf-cell, and the pressure of the air surrounding the leaf-cell.

Thus, Forces causing movement downwards = $p + T + Q$.

The total force tending to cause movement of water between the stem and leaf will therefore be

$$P + q - p - T - Q,$$

and the force will be upwards when this expression is positive and downwards when it is negative. When this expression is positive it will moreover represent the leaf-pull. (The same expression is true for case a .)

If, then, C = the conduction rate in the stem

and R = the resistance to flow of the whole system,

C may be put = $\frac{\text{Force causing flow}}{\text{Resistance to flow}} = F/R$ (cf. Köckemann, 5),

$$\text{where } F = P + q - p - T - Q.$$

The conduction rate, C , is then given by

$$C = \frac{(P - p) + (q - Q) - T}{R},$$

the flow being upwards when this value is positive and downwards when it is negative. Moreover, when the flow is upwards the rate of conduction will be directly proportional to the leaf-pull or foliar forces raising the water. It is to be noted also that under any given pressure, Q , it becomes equal to the rate of transpiration, when such a condition is reached that the latter maintains a constant value of $P - T$, the suction pressure of the leaf-cells, that is when the leaf-cells have attained a constant equilibrium volume under the pressure applied. This follows, since all the terms in the expression other than P and T are constant, and different transpiration rates will merely lead to different definite values of $P - T$ and leaf-cell volume in dynamic equilibrium with each particular rate.

It will be observed from the above expression for C that it is equivalent

to saying that the force causing conduction is equal to the difference between the suction pressures of the leaf-cell and tract less the pressure deficit, since the equation can also be written

$$C = \frac{(P - T) - (p - q) - Q}{R}.$$

This equation will be referred to again in the following paper.

The excess pressure applied to the leaf surface may be regarded as reducing the suction pressure of the leaf-cells by being added to the turgour pressure, or as opposing the action of the true suction pressure as given by $P - T$. When the excess pressure, Q , is applied to the surfaces of the leaf-cells, the effective suction pressure becomes only $P - (T + Q)$ or $(P - T) - Q$, in just the same way as the 'suction pressure' of the tracts becomes increased during conditions of tension in the tract to $(p - q)$. The value of q being negative during tension, the suction pressure of the tract would become very large, and produce the same results as the excess pressures in the experiments. In any case, the forces causing movement of water depend only upon the relation of $(P - T - Q)$ to $(p - q)$, and the rate of conduction is decided by the same equation as that given above. So far as the pressure deficit factor is concerned, therefore, the effect only depends upon the value of the quantity $(q - Q)$, the difference in pressures, and not upon the absolute values of either q or Q .

It follows, therefore, that since the absorption rates as measured in the experiments are actually measures of the conduction rate, C , they are true measures of the effects which changes of pressure deficit produced in nature will have upon the foliar forces causing conduction and upon transpiration rate, although these changes in nature are produced by alterations in q , the tract pressure, instead of alterations in the pressure at the leaf surfaces. When alterations in volume of the leaf-cells are not taking place, moreover, they will be actual measures of the transpiration rate.

EXPERIMENTAL METHODS.

The branch was enclosed in a pressure cylinder as in the experiments of Dixon and Ball (2) and Chibnall and Grover (1), the cylinder, however, in this case being of cast iron. The rate of absorption of water at atmospheric pressure by the protruding base of the branch was measured with different increased pressures in the cylinder. Further details, therefore, come under the headings of 'Methods of Measuring the Absorption Rate' and the construction of the cylinder with which the pressure was applied.

Methods of Measuring Absorption Rate.

Three different methods have been used for the measurement of the rate of absorption, as follows:

(i) The base of the branch was fitted into a simple potometer and the rate of travel of a water meniscus in a graduated capillary tube was followed with a stop watch;

(ii) the base of the branch was fitted into the vessel, A, of a self-recording potometer, recording in $\frac{1}{200}$ ths of a c.c., as described by the writer in an earlier paper (4); and

(iii) the base of the branch was allowed to take up eosin solution, the rate of penetration being estimated at intervals by cutting off a few cm. from the end of the branch under water and rapidly sectioning back to find the distance travelled by the eosin in a given time interval. In a variation of this method the travel of the stain in the superficial tracts of a part of the branch from which the bark had been stripped was observed for certain purposes in certain cases.

Potometer methods are open to the objection that when the branch is enclosed in the pressure cylinder and relatively high pressures are used, air becomes forced into the plant via the stomata, down the stem through the intercellular space systems of the cortex and pith and leaks from the cut base of the stem into the potometer vessel, thus vitiating the results. This difficulty, however, can be overcome and useful results obtained with potometers, especially the self-recording instrument referred to above, by the device of ringing the branch at the level of the seal through the base of the cylinder, to prevent leakage of air down the cortex. To improve matters still further, the pith may also be excised for a length of about 1 cm. through a small lateral slit in the wood at the same level as the ring, the cavity being afterwards filled with soft wax. If this dissection be carefully performed, reliable results may be obtained with pressure deficits up to ten atmospheres, but the method is unnecessarily laborious in cases where the eosin method can be substituted. The eosin method was therefore found to be the most generally useful.

The Construction of the Pressure Cylinder.

Diagrams of the pressure cylinder and accessories are given in Fig. 2.

The pressure cylinder consists of a body, AA, and two ends, BXXB and CC, mounted on supports, DD, on a wooden base, JJ, measuring 3 ft. \times 2 ft. \times 1½ in. This base, JJ, also carries a control valve, R, a relief valve, O, a release valve, Q, and a pressure gauge, P, mounted on a steel block, S, on the supply pipe, TT, through which air is supplied from an ordinary 'B.O.C.' gas cylinder at a pressure of 100–120 atmospheres. The base, JJ, also carries the potometer (not shown) mounted in front of the valve block when in use.

The body, AA, of the cylinder is of cast iron. The inside height of the body is 9 in., the inside diameter 6 in., the thickness of the walls 1½ in. The lower flange is 1½ in. thick, and the upper flange, which is more deeply

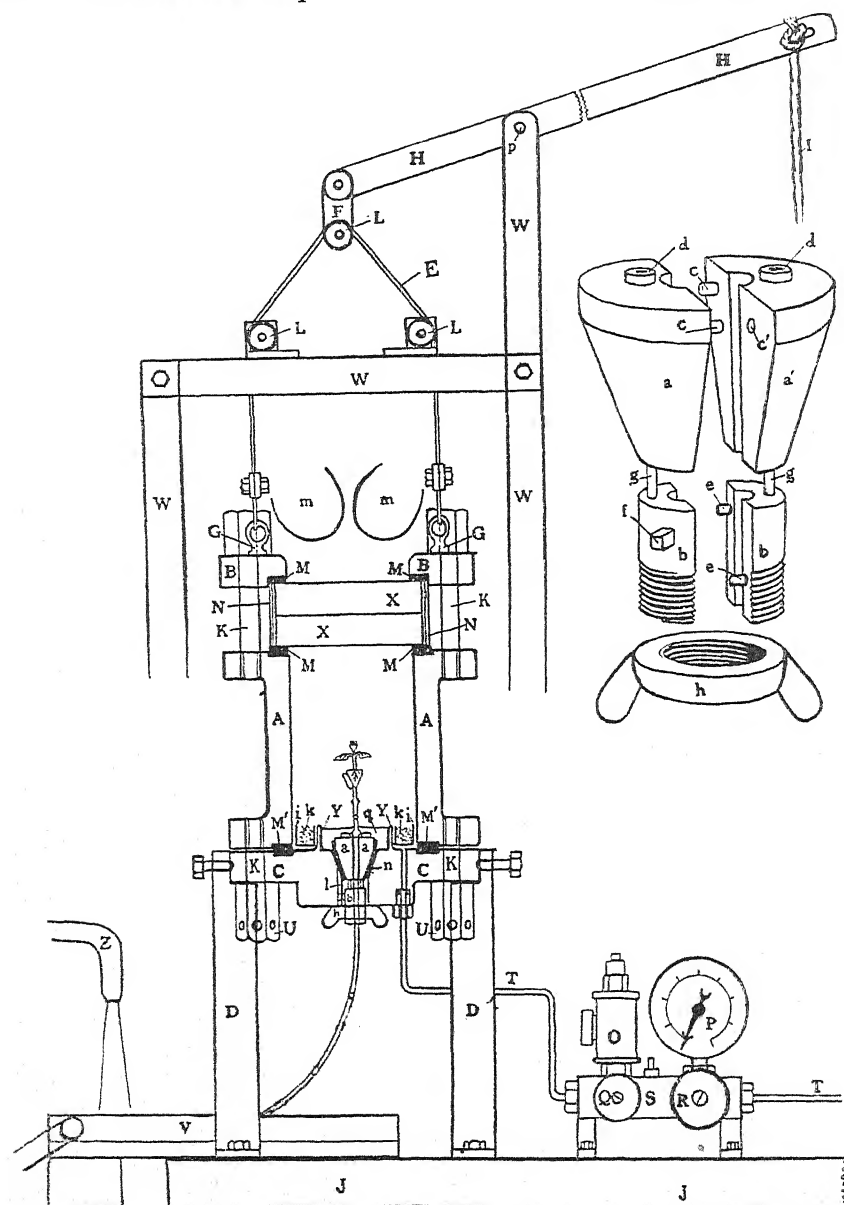


FIG. 2. Diagram of elevation of pressure cylinder with lifting gear and accessories. To avoid confusion the bases of the lifting gear supports which actually bolt on to the same baseboard, JJ, as the rest of the apparatus are not shown. In practice, moreover, the lifting gear is mounted in the vertical plane at right angles to that indicated. Inset on right: The plug for platform seal. AA = body of cylinder (cast iron). BB = cast iron flange of top of cylinder. CC = cast iron base of cylinder, or platform. DD = supports of platform (4 altogether). E = steel cable for lifting. F = link. GG = eyes for lifting cable. HH = lifting arm. I = rope for platform lifting gear. JJ = base board. KKKK = studs. LLL = pulleys for cable. MMMM = rubber washers in cylinder head. M'M' = rubber washer on platform. NN = brass shield. O = relief valve. P = pressure gauge. Q = release valve. R = control valve. S = steel block carrying valves. T = supply pipe.

recessed for a rubber washer $1\frac{1}{8}$ in. Each flange is fitted with eight $1\frac{1}{8}$ in. studs with nuts for the attachment of the ends. The upper end is kept permanently attached: the lower end is detached for inserting plants, &c., by taking off the nuts, UU, the whole cylinder then being lifted clear of it by the lifting gear above. By means of this it can be fixed suspended with a clearance of about 9 in., while a plant is being inserted through the base and sealed in for an experiment. The lifting gear consists in a steel lever, HH, 4 ft. long, pivoted at *p* on supports, WWWW, and carrying a link at F bearing a steel pulley, over which passes a steel cable attached to eyes, GG, on the upper end of the cylinder. The long arm of the lever comes about 8 ft. from the laboratory floor, and is therefore operated by a rope, I. The inside of the body of the cylinder is enamelled white in order to improve and equalize the illumination (see below).

The upper end of the cylinder consists in two discs of plate glass, XX, 7 in. in diameter, and each $1\frac{1}{2}$ in. thick. These are cemented together with clear balsam to form a single plate 3 in. thick, a single piece of the necessary thickness being unobtainable. The plate is held in position by a cast iron flange, BB, $1\frac{1}{8}$ in. thick, and fixed by eight $1\frac{1}{8}$ in. studs with nuts to the top flange of the cylinder. Above and below the glass plate are rubber washers, MMMM, $\frac{1}{8}$ in. thick, fitting into recesses $\frac{3}{8}$ in. deep in both flanges. A brass shield, NN, $\frac{1}{16}$ in. thick and 3 in. deep, surrounds the glass plate in order to prevent blowing out of the washers. The surfaces of the washers were lightly coated with jointing cement before assembly, and with occasional re-tightening of the nuts no difficulty was experienced with leakage up to pressures of 75 atmospheres.

The lower end, CC, of the cylinder is of cast iron, and is referred to as the platform. It is readily detachable from the body, and bolted permanently to the supports, DD, by which it is given a clearance of approximately 10 in. from the wooden base. It was found in practice that though this construction was convenient for most types of experiment, slightly more clearance would have been advantageous in some cases. The bottom end or platform is fixed to the cylinder body in the same manner as the upper end by eight $1\frac{1}{8}$ -in. studs and nuts. The nuts here, however, are provided with holes for a $\frac{3}{8}$ -in. tommy-bar for more rapid handling. By this device the cylinder could be opened or shut in one minute. A rubber washer is placed between the cylinder flange and the platform, the cylinder flange

UU = bottom nuts with provisions for tommy bar. V = water trough. WWWW = supports for lifting gear of 3 in. barrel and 4 in. channelling. XX = glass window in top of cylinder. VV = cup for wax seal. Z = movable tap. aa' = conical oak split plug. bb = threaded brass cylinder of plug. cc = locating pins. c' = hole for locating pin. dd = nuts on upper ends of gg. ee = locating pins on brass part. f = key to prevent turning in platform on screwing up h. gg = steel rods connecting bb to aa'. h = wing-nut drawing plug into seating in platform. ii = two half-annular trays for calcium chloride. kk = calcium chloride. l = slot in hole in platform for key, f. mm = positions of electric light bulbs during experiments. The bulbs are mounted on movable arms (not shown). n = rubber washer round conical plug. p = pivot of lifting lever. q = wax seal. For dimensions and further description see text.

being recessed for it $\frac{1}{32}$ in. deep and the platform $\frac{3}{8}$ in. deep. The washer remains in the deeper groove (in the platform) on opening the cylinder, being also fixed in this groove by a little jointing cement. The platform is $1\frac{1}{2}$ in. thick at the edges and $2\frac{3}{8}$ in. thick in the middle. The upper surface of the casting bears an annular projection, *yy*, 1 in. deep and $\frac{1}{8}$ in. thick, and with an inside diameter of $3\frac{1}{2}$ in. surrounding a central hole, and forming a trough for a wax seal. The plant is sealed through the hole in the platform by means of a special split wooden plug, which fits into the conical upper part of the hole. A separate diagram of the plug on a larger scale is given in Fig. 2 (inset). The conical upper part, *aa*, is of oak wood. The faces are caused to fit accurately together by two small pegs, *cc*, which fit into corresponding holes in the opposite faces. The central hole in the plug when assembled is $\frac{1}{4}$ in. in diameter. The halves of the split plug are each connected below with the two halves of a split brass cylinder, *bb*, by $\frac{1}{8}$ -in. steel rods running through them, the rods being screwed into the brass pieces below and carrying nuts, *dd*, at the upper ends. The faces of the brass parts are also caused to fit accurately together by projecting pins, *ee*, fitting into corresponding holes in the opposite faces, the central hole when the halves are fitted together also being $\frac{1}{4}$ in. in diameter. The split brass cylinder slides easily into the lower part of the hole through the platform, and is prevented from turning round in it by a short key, *f*, on the outside of the brass cylinder, fitting into a longitudinal groove, *l*, on the inside of the hole in the platform. The lower end of the brass cylinder just projects below the platform, and is threaded to carry a wing-nut, *h*, by means of which the conical wooden plug can be pulled down tightly into the conical upper part of the hole in the platform. In use, the two complete halves of the plug are fitted together round the stem of the plant with a small quantity of soft, warm wax in the central groove and on the joining faces; a short length ($1\frac{1}{2}$ in.), of rubber washer, *n*, made from a piece of $1\frac{1}{2}$ -in. bicycle inner tube is slipped over the conical wooden part of the plug; the base of the stem and then the plug are passed through the hole in the platform, with the cylinder raised by the lifting gear; the wing-nut, *h*, is passed over the base of the stem from below, and screwed on to the protruding brass part of the plug to pull the conical part tightly into its seating, and the trough is filled with molten wax mixture at 40° C. Throughout the operation of sealing the plant through the platform the end of the stem is kept in a small specimen tube full of water which will readily pass through the hole in the platform and also through the wing nut, *h*. The plant never, therefore, needs to be taken out of water at all. The wax used throughout is a mixture of equal parts of paraffin wax and resin in which has been dissolved approximately one-sixth of the quantity by weight of india rubber. A little vaseline was added if necessary to cause the mixture to remain liquid enough for sealing at 40° C.

The Inlet and Pressure Control.

The inlet tube by which air under pressure is admitted to the cylinder is fixed into the lower surface of the platform, CC, through a union with a fibre washer, the tubing used being of drawn steel with an inside diameter of $\frac{1}{8}$ in., and outside, $\frac{3}{8}$ in. The supply was from an ordinary B.O.C. gas cylinder of capacity 20 cu. ft. at 120 atm. As the actual volume of the supply cylinder was therefore $\frac{1}{3}$ cu. ft., and the volume of air in the experimental cylinder when in use approximately $\frac{1}{8}$ cu. ft. (at atm. press.), this allowed pressures up to 60 atm. to be obtained on one charge. The supply cylinder was recharged from a compressor in the Engineering Department of the College, and could, if necessary, or to obtain higher pressures, be recharged during experiments without releasing the pressure in the experimental cylinder. This was done by shutting off the control-valve, R, before detaching the supply cylinder. This valve is incorporated in the steel block, S, mounted on the base board, which also carries a Bourdon Pressure Gauge, P. The relief valve, O, could be set to blow off at any desired pressure. The release valve, Q, is provided in order that the pressure in the experimental cylinder may be released without detaching the supply cylinder. The cylinder set up with a plant in it as during experiments was tested hydraulically at a pressure of $\frac{1}{2}$ -ton/sq. in. (75 atm.)

The air. The air as supplied from the compressor was of approximately the same humidity as the laboratory air. Control experiments showed that absorption continued at the same rate whether the cylinder was filled with laboratory air or air from the supply cylinder. The rates in either case, however, fell off if no drying agent were enclosed in the experimental cylinder. To prevent this and maintain the humidity of the enclosed air roughly constant two semi-lunar vessels of anhydrous calcium chloride, *kk*, were placed inside the cylinder on the platform, surrounding the central wax-seal trough. With this device the evaporation rate of a branch enclosed in the cylinder remained constant throughout a period equal to that of the duration of the experiments. This was shown by controls (p. 226). The presence of the chloride was therefore considered to be an effective measure for maintaining sufficiently constant humidity in the cylinder.

The different forms of potometer when in use were mounted on the base-board in front of the valve block, but are not shown in the diagram. When potometers were not employed, and the eosin method was used, the end of the branch which was much longer (e.g. 60 cm.) than in the other cases, was allowed to pass into a metal trough, V, of running water from a movable supply, Z, under which the successive portions could be detached for the measurements of the rates of penetration of the eosin.

The Preparation of the Branch.

The branch to be used was cut from the tree under water by cutting in the middle of the bent lower part of the branch while held under water in a trough. The trough and branch were then brought into the laboratory and the branch transferred to a larger trough, the small one being immersed in water in the larger one during the transference. The whole branch (about 60 cm. long) was then immersed in the water in the larger trough with the exception of the top part bearing the last two to eight leaves according to the species and size of the leaves, and, while immersed, all the lower leaves were detached with a sharp scalpel. After allowing the branch to remain a further five minutes in the water it was taken out, and the whole of the lower part from which the leaves had been detached was thoroughly coated with vaseline, which was also specially rubbed into the wounds left by the detachment of the lateral branches and leaves. The base of the branch was then placed in a small specimen tube full of water, and the split plug was fitted to the zone immediately below the remaining leaves at the apex in the manner already described. The branch was then transferred to the cylinder, the plug pulled down by screwing on the wing nut, the base of the branch placed under running water in the trough, v, and the specimen tube removed. A charge of dry calcium chloride was then placed in the semi-lunar trays, and these fitted in position on the platform and the cylinder lowered and bolted down. Two 100-watt lamps were switched on in the positions, *mm*, immediately above the plate-glass window in the top of the cylinder, and the whole was left for an hour before beginning to take readings. The illumination was shown to be sufficient to keep the stomata open in all cases by the injection method (δ), and the interval for the attainment of a steady natural absorption rate was shown to be sufficient by control experiments (see Series I, below).

THE TYPES OF EXPERIMENT PERFORMED.

The experiments by the Pressure Cylinder method may be divided into six series according to the objects of the experiments and the methods of procedure adopted, as follows:

Experiments by Potometer Methods, (i) and (ii), see p. 219.

Series I. Control and preliminary experiments, including experiments with evaporation models and with plants at atm. pressure.

Series II. Miscellaneous preliminary experiments indicating the general nature of the effects of changes of pressure deficit on absorption rate.

Series III. Systematic potometer experiments showing the changes

in rate of absorption which accompany a gradually increasing pressure deficit.

Experiments by the Eosin Method, (iii), see p. 219.

Series IV. Systematic eosin method experiments showing the changes in rate of absorption which accompany a gradually increasing pressure deficit.

Series V. Systematic experiments showing the changes in absorption rate taking place during periods of sustained constant pressure deficits of different magnitudes, and various experiments demonstrating the effects of reduction of the pressure deficit.

Series VI. Experiments to show the relative parts played by changes in the transpiration rate and in the degree of mechanical compression of the leaf-cells on the rate of absorption.

The experiments of these series will be described in the order given, Series I and II in the present communication, and the others in a later paper.

Since individual experiments necessarily occupied a considerable time, especially in the setting up and preliminary preparations, the guiding principle throughout has had to be to secure the greatest possible precision in individual experiments rather than a larger number of experiments, the results of which could have been averaged and subjected to statistical treatment. Many experiments of one kind on one type of plant could only be performed once, but where any reading or whole experiment did not appear to be in conformity with the neighbouring readings or experiments of its series, the experiments were always repeated until a series of convincing concordant readings was obtained. This applies throughout both to the control experiments and to those of the later series.

SERIES I.

Control and Preliminary Experiments.

This Series is sub-divided into three groups of experiments, *A*, *B*, and *C*, for different purposes or conducted by different methods.

Group A. Experiments to prove the adequacy of the illumination. The usual length of time for which plants were kept in the cylinder during experiments was approximately two hours. Accordingly, leaves of each kind of plant used were tested immediately after cutting the branch from the tree by means of the injection method (6) for the size of the stomatal apertures. The branch was then put up in the cylinder in the same way as for the later experiments, and after a period of $2\frac{1}{2}$ hours taken out again and immediately re-tested. All the experiments were repeated several times with the absolutely uniform result that the extent of injection of the leaves

which had been $2\frac{1}{2}$ hours in the cylinder was invariably exactly the same as that shown by fresh untreated leaves. The results are given below in Table I, — signifying no injection, + signifying slight injection, and ++ very rapid injection of the intercellular spaces.

TABLE I.

	Absolute alcohol.		Xylol.		Petrol ether.	
	Fresh leaves.	Leaves from cylinder.	Fresh leaves.	Leaves from cylinder.	Fresh leaves.	Leaves from cylinder.
Acer	—	—	+	+	++	++
Aesculus	—	—	—	—	++	++
Ligustrum	—	—	+	+	++	++

The stomatal response would be far too slow to allow of the stomata opening as a result of taking out of the cylinder in time for the test, and it was therefore concluded that the illumination was sufficient to cause the stomata to remain open in the cylinder, a point which was further proved by the control experiments of Group B.

Group B. The adequacy of the illumination was also proved in a series of experiments, using *Aesculus*, by means of potometer readings.

Aesculus branches were set up in the cylinder with no drying agent, and simple potometer readings were taken at one minute intervals. These showed a falling off in the absorption rate for the first 40 minutes, evidently on account of the increasing humidity of the air, the average readings for the first, second, third, and fourth ten-minute periods in a representative example being 0.59, 0.46, 0.42, and 0.39. The rate then became approximately constant for the next 2 hours at 0.38. At the end of this time the cylinder was opened and raised slightly to allow the circulation of drier air round the branch, *further illumination, however, not being admitted*. In the first, second, and third ten minutes after opening the absorption rates were 0.38, 0.55, and 0.64 respectively, showing that a value slightly above the original one could be rapidly attained *without any increase in the illumination*, and the stomata could not therefore have closed. This type of experiment was repeated several times with similar results.

Group C. To prove the efficiency of the drying agent. In this group of experiments controls were performed to show that the falling off in absorption rate noted in the above experiments (Group B) did not occur if the usual supply of anhydrous calcium chloride were present in the cylinder.

It was shown both by experiments with plants and also with porous pot atmometers in the cylinder that the absorption rate could continue constant hour after hour, or even in certain cases might become greater and greater with time. These showed the adequacy of the drying agent. Representative experiments are quoted below.

Experiment 1 (Group I. C). The plant used was *Eupatorium adenophorum*, and the illumination two ' $\frac{1}{2}$ -watt' 100-watt lamps approximately 9 in. from the leaves. The plant was mounted in the usual way with calcium chloride in the cylinder, and the absorption rate was followed up by means of a simple potometer continually for the first seven hours, and intermittently for seventeen days after placing in the cylinder.

TABLE II. (Experiment 1.)

Hours after placing in cylinder	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.
Rates of absorption	361	411	405	399	390	387	385

The results for the first seven hourly periods are shown in Table II, and show an increase at first, followed by a slow falling off.

It is to be noted that in normal experiments, as will be seen later, only the second and third hours were used, as the plant was left for one hour in the cylinder after setting up without taking readings. It would appear then from the above readings (Table II) that the maximum error to be expected during this hour is approximately $(411-405)/411$ per cent., or less than $1\frac{1}{2}$ per cent. A number of other similar control experiments also showed that the rate was invariably most constant during the second and third hours, and these were therefore always chosen for experimental readings. The error during these hours was never greater than 2 per cent., and in many cases with *Acer* and *Aesculus* no variation could be detected at all (cf. Experiment 2 of Series I. C, Table III). It is significant in the experiment quoted above (I. C. 1, Table II) that the rate of absorption increased in the second hour as compared with the first. This was often the case when the branch used had previously been exposed to rather poor illumination, and goes further to prove that neither light nor humidity limits the rate of transpiration in the cylinder.

In order to test the ultimate effects of the confined atmosphere of the cylinder on the plant, the plant in this experiment was left in the cylinder without any artificial illumination, weak daylight alone entering, as the apparatus was left on the bench in the laboratory, for three days. The absorption rate had then fallen to 222 in the same units as above (Table II). After a further three days it had fallen to 139, at which point readings of absorption were discontinued. On the eleventh day (from the beginning of the experiment), however, the plant still appeared to be perfectly healthy when inspected through the glass in the upper end of the cylinder. Not until the twelfth day of the experiment did it show visible signs of failing, but on this day it appeared to have become slightly flaccid. On the seventeenth day it first showed signs of dying back from the tips of the leaves, the older leaves being completely wilted. Since the plant can

remain healthy in the cylinder for several days, however, the above account being typical of a number of similar experiments, it is evidently reasonable to assume that it does not suffer appreciably from the conditions imposed upon it in the course of an ordinary experiment. The other plants used fared very similarly, *Acer* and *Aesculus* branches remaining healthy for 4-6 days, and showing no pronounced falling off in absorption rate until after the second day in the cylinder. Experiment showed, however, that, in order that humidity should not become limiting, it was desirable that the actual evaporating surface should not exceed approximately 10 sq. in., or 65 sq. cms. In experiments, therefore, only so many leaves were left attached as made the active surface roughly 6-8 sq. in. (or 40-50 sq. cm.). The same was found to be true of evaporation models. Whereas a small plaster of paris plug in a thistle funnel would show a constant rate of evaporation hour after hour, if a large porous pot were used the rate fell off for the first half hour or so to a much smaller constant value evidently limited by the humidity of the air.

Readings with the automatic potometer (4) in further experiments of this series, which need not be recorded in detail, showed that the absorption rate with all the different plants used almost invariably fell off during the first hour if the branch were placed in the cylinder as quickly as possible after cutting from the tree. This was evidently to be ascribed to the fact that the branches were almost invariably collected in circumstances or at times of the day when a deficit would have been originally present in the branch when cut, and this evidently required from $\frac{1}{2}$ -1 hour to become completely eliminated. The most satisfactory procedure was, therefore, to leave the branch standing in water for half an hour after cutting and then set it up in the cylinder and allow a further period of 1 hour to elapse before beginning to take readings. This procedure gave the most uniform results, and was therefore always adopted.

An experiment on *Aesculus* is quoted below to show the effect of placing the branch in the cylinder immediately after cutting and the consequent falling off of the readings. It will be observed, however, that the readings became perfectly constant after the first hour and a quarter (thirty-ninth 2-minute reading) (Table III). In other cases the readings would not become constant until $1\frac{1}{2}$ hours after setting up, but once constant, they remained so for at least $1\frac{1}{2}$ hours in most cases, and frequently for over 2 hours. The readings were obtained with the automatic self-recording potometer (4), and as given here, represent the mean absorption rates for successive intervals of 2 minutes. (The units are $\frac{1}{100}$ ths of a c.c.)

That the falling off during the first hour in experiments such as that quoted above (Table III) was definitely only due to the satisfaction of an original deficit, and not to humidity or lack of light, was further shown by the fact that there was in no case any increase in the absorption rate when

the cylinder was opened at the end of the experiment. Moreover, if time (e.g. $1\frac{1}{2}$ hours) were allowed for the satisfaction of the original deficit before placing the branch in the cylinder, the absorption rate remained sensibly constant for the first $1\frac{1}{2}$ -2 hours after setting up in all cases. The following result with *Aesculus* is typical of a number of controls performed in this way with each kind of material used (Table IV).

TABLE III. (Experiment 2)

Mean Absorption Rates by Aesculus Branch for 2-minute Intervals.

1st $\frac{1}{2}$ hr.	6.0	6.0	6.0	5.5	4.2	4.5	4.8	5.3	4.4	4.4	4.0	4.0	4.0	3.6	3.8
2nd $\frac{1}{2}$ hr.	3.6	3.6	3.2	3.2	2.8	3.0	3.0	3.0	2.8	2.6	2.6	2.6	2.6	2.5	2.5
3rd $\frac{1}{2}$ hr.	2.5	2.5	2.4	2.3	2.3	2.3	2.3	2.2	2.0	2.0	2.0	2.0	2.0	1.9	2.0
4th $\frac{1}{2}$ hr.	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

TABLE IV.

Readings of Absorption Rate of Aesculus Branch expressed as averages for successive 10-minute periods in cylinder, after allowing $1\frac{1}{2}$ hours in water previous to setting up.

3.2	3.1	3.1	3.1	3.1	3.1	3.1	3.0	3.1	3.1	3.1	3.1	3.0	3.1	3.1	3.1	3.1
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(The experiment was continued for three hours.)

Experiments of these types and also the behaviour of the absorption rates in many of the later experiments (notably Experiments 14, 16, 21, 22 of Series II, &c.) abundantly showed that neither light nor humidity caused any decrease in the rate of absorption during periods equal to the lengths of the ordinary pressure experiments later described.

A larger number of further controls establishing the same points were performed by the eosin method and will be quoted in the following paper. (See Controls to Series IV.)

Group D. Experiments to demonstrate that no damage was done by the method of sealing the plant in the cylinder. The possible effects of the warm wax used for sealing upon the stem were investigated, but in no case, even with the more vulnerable plants such as *Eupatorium*, could any traces of slime or other blockage in the conducting tracts be detected by staining with saffranin, &c., although the walls were always densely stained.

SERIES II.

Miscellaneous Experiments by the Potometer Method, indicating the General Nature of the Relation of Absorption Rate to Changes in the Pressure Deficit.

Experiment 1. Readings of the absorption rate of *Aesculus* were taken at 2-minute intervals with the simple potometer, the pressure

raised and lowered to different values at intervals. The results are given in Table V, where all the columns represent one continuous experiment.

TABLE V.

Press. ¹ lb./in.	Rate.	Press. lb./in.	Rate.	Press. lb./in.	Rate.	Press. lb./in.	Rate.	Press. lb./in.	Rate.
0	6.5	15	2.5	15	2.3	0	3.7	0	4.0
0	5.9	0	10.0	15	2.4	0 ²	3.6	0	3.8
0	5.3	0	10.0	15	2.5	0	3.5	0	3.2
0	3.5	0	9.0	15	2.7	0	3.4	0	3.1
0	3.4	0	8.3	15	3.2	0	3.2	0	2.6
0	3.4	0	8.3	15	3.3	0	3.0	10	0.7
0	3.4	0	6.3	0	12.5	0	3.1	10	1.7
0	3.4	0	6.3	0	11.1	0	3.2	10	2.0
30	0.0	0	5.3	0	11.1	10	0.5	10	1.5
20	0.0	0	5.3	0	10.0	10	1.6	10	1.5
15	0.1	0	4.6	0	9.0	10	2.0	0	5.0
15	2.2	0	4.6	0	8.3	10	2.0	0	4.8
15	1.9	0	4.6	0	7.7	10	2.0	0 ³	5.0
15	2.3	0	4.2	0	6.7	0	10.0	0	5.0
15	2.5	0	4.2	0	5.5	0	6.0	0	5.0
15	2.3	15	1.5	0	4.8	0	5.0		
15	2.5	15	2.1	0	4.4	0	4.0		

(For discussion of these results and conclusions, see pp. 233, 234, 237.)

Experiment 2. In this experiment readings of the absorption rate of *Eupatorium* were taken against time by means of the simple potometer—

(a) On the fresh plant.

(b) After exposure to a pressure of 150 lb./in. (10 atm.) for 25 minutes.

(c) After subsequent exposure to 40 lb./in. for 4 minutes.

(d) After further exposure to 20 lb./in. for 3 minutes.

(e) After further exposure to 20 lb./in. for 9 minutes.

(f) After further exposure to 10 lb./in. for 3 minutes.

(g) After a final exposure to 60 lb./in. for 13 minutes.

All the actual readings were taken with atmospheric pressure in the cylinder in the intervals between these successive exposures to pressure. The lengths of the intervals will be apparent from the table. The results are shown in Table VI, and serve to show how the absorption rate at atmospheric pressure varies according to the pressure previously experienced.

The final period at 60 lb./in. caused all the leaves, except the youngest and the edges of the older ones, to become injected with water. The total length of the experiment was 24½ hours. For further discussion, see pp. 233, 234.

¹ It is to be noted that throughout these experiments the pressure deficit is equal to the pressure applied, as the gauge only measures the excess above atmospheric pressure.

² At this point the experiment was switched over to the automatic potometer and continued in the same units.

³ At this point the cylinder was opened to the air.

TABLE VI.

Showing Absorption Rates by Eupatorium at Atm. Pressure After Periods of Exposure to Different Pressures. The Times Given are in Minutes from the Beginning of Each Interval.

(a) After 60 minutes at atm. pressure	{	Time	0	8	14	23	43	88	93	minutes.
	{	Rate	2.9	3.0	3.0	3.0	3.1	3.3	3.3	units.
(b) After 25 minutes at 150 lb./in.	{	Time	0	2	8	11	15	20	25	43 minutes.
	{	Rate	10.0	7.3	5.2	4.6	4.5	4.3	4.3	3.3 units.
(c) After 4 minutes at 40 lb./in.	{	Time	0	2	12	20	72	77	83	88 93 minutes.
	{	Rate	8.3	7.0	4.8	4.4	4.1	3.9	3.8	3.75 3.70 units.
(d) After 3 minutes at 20 lb./in.	{	Time	0	3	6					8 minutes.
	{	Rate	6.6	6.6	8.3	10.0				units.
(e) After 9 minutes at 20 lb./in.	{	Time	0	2	4	9	16	19	23	34 41 51 54 minutes.
	{	Rate	15.1	11.1	8.3	5.9	5.0	4.8	4.5	4.2 4.1 4.0 3.9 units.
(f) After 3 minutes at 10 lb./in.	{	Time	0	5	6	9	13	23	30	33 38 63 minutes.
	{	Rate	6.6	7.2	6.9	5.7	5.0	4.2	4.2	3.8 3.8 3.6 units.
(g) After an interval of 14 hours the pressure was raised to 60 lb./in. for 13 minutes. Subsequent rate = 2.2.										

Further experiments were made upon *Acer* and *Aesculus* in which the effects of changes in the pressure deficit upon absorption rate were followed up by means of the automatic potometer (4). The results of some shorter experiments on *Acer* and *Aesculus* are given in Tables VII and VIII, in which the absorption rates at different pressure deficits are given as averages for successive 5-minute intervals. The results of longer experiments on *Aesculus* are given in Tables IX, X, and XI (Experiments 21, 22, and 23). In these the rates recorded are means for successive periods of 2 minutes, also obtained with the self-recording potometer.

The results are discussed on pp. 233, 234, 236, 237.

Fresh material was of course used for every experiment.

TABLE VII.

Giving Mean Absorption Rates for Successive 5-minute Intervals at Different Pressure Deficits as Indicated.

Material: *Acer*. Method: Self-recording potometer.

Experiment 3.		Experiment 4.			
Pressure.	Rate.	Pressure.	Rate.	Pressure.	Rate.
0	4.1	0	3.3	0 ¹	14.3
5	2.5	0	3.3	0 ²	11.1
5	2.7	0	3.3	0	7.7
0	4.5	100	0.0	0	6.3
0	3.6	100	0.0	0	3.1
		100	0.0	0	2.7
		100	0.0	0	2.5

¹ The pressure of 100 lb./in. was maintained for a period of 1 hour before taking this reading.

² An interval of half an hour was allowed before taking this reading.

TABLE VIII.

Giving Mean Absorption Rates for Successive 5-minute Intervals at Different Pressure Deficits as Indicated.

Material: Aesculus. Method: Self-recording potometer.

Expt. 5.		Expt. 8.		Expt. 12.		Expt. 15.		Expt. 18.	
Press.	Rate.	Press.	Rate.	Press.	Rate.	Press.	Rate.	Press.	Rate.
0	3.1	0	0.2	0	2.8	0	5.3	0	1.7
5	0.9	12.5	0.1	20	1.0	0	4.6	45	0.6
5	2.0	0	1.7	0	5.0	0	3.8		
5	2.2			0	3.5	0	3.2		
5	2.5			0	2.2	0	2.8		
		Expt. 9.		0	1.8	0	2.5	Expt. 19.	
		Press.	Rate.	0	1.5	0	2.3	Press.	Rate.
Expt. 6.		0	1.7			0	2.1	0	2.8
Press.	Rate.	12.5	0.7	Expt. 13.		0	2.0	50	—
0	0.8	12.5	1.0	Press.	Rate.	0	2.0	50	0.9
0	0.8	12.5	1.0			0	2.0	50	1.0
5	0.6	12.5	1.0	0	2.6	25	1.3	50	1.1
0	1.4	12.5	1.0	0	2.6	25	1.9	50	1.3
0	1.1	12.5	1.0	20	1.2	25	1.5	50	1.3
0	1.0	0	1.7	20	1.3	25	1.4	50	1.4
0	0.8			20	1.6	25	1.3	50	1.5
0	0.8	Expt. 10.		20	1.6	0	3.7	0	8.5
0	0.8	Press.	Rate.	20	1.6	0	2.8	0	4.1
0	0.7			0	16.6	0	2.5	0	3.1
0	0.7	0	1.5	0	12.5	0	2.2		
		0	4.4	0	4.4			Expt. 20.	
		15	0.9	0	3.8	Expt. 16.		Press.	Rate.
Expt. 7.		0	3.2	0	3.6	Press.	Rate.	0	6.1
Press.	Rate.	0	2.1	0	3.4			0	5.5
0	1.4			0	3.1	0	1.8	0	5.5
0	1.3	Expt. 11.		0	3.0	30	0.4	0	5.5
0	1.3	Press.	Rate.	0	2.9	0	1.9	100	0.0
0	1.3	0	5.5	0	2.9			0	0.0
0	1.3	0	3.3	0	2.9	Expt. 17.		0	6.6
0	1.4	0	3.0			Press.	Rate.	0	6.0
0	1.3	0	3.0	Expt. 14.				0	5.0
0	1.3	15	1.8	Press.	Rate.	0	1.7	0	5.0
0	1.3	15	1.8			30	0.7		
0	1.3	15	2.3	0	3.1	30	0.8		
10	0.2			25	1.8	30	0.8		
10	0.7			25	2.0	30	0.75		
10	1.0								
10	0.8								
10	0.8								

¹ See footnote 1, p. 230.

CONCLUSIONS FROM SERIES II.

Although rather less importance is attached to the actual *numerical* results of the foregoing series than to those of the later series (owing to the absence of any guarantee that the leakage error to which all potometer methods are necessarily subject when used for this type of experiment (p. 219) has been completely excluded), the following general conclusions are evidently justified:

TABLE IX. (Experiment 21.)

Giving Mean Absorption Rates for Successive 2-minute Intervals at Varying Pressure Deficits as Indicated. The Readings were obtained with Aesculus, using the Automatic Potometer.

Press. ¹	Rate.	Press.	Rate.	Press.	Rate.	Press.	Rate.	Press.	Rate.
0	9.0	0	9.0	0	11	100	0	5	3.3
0	8.5	0	8.5	0	8.0	0	12	5	3.5
0	8.5	0	7.0	0	6.5	0	8.0	5	3.8
30	0.0	0	5.8	0	5.8	0	6.0	5	3.9
30	0.0	0	5.8	0	5.0	0	5.6	0	7.0
0	9.3	0	5.5	0	4.6	0	5.2	0	4.8
0	8.8	0	3.8	10	0.75	0	5.1	0	4.4
0	7.2	100	0.0	10	2.5	0	5.0	0	4.2
0	6.2	0	18	10	4.3	5	1.25	0	4.0
20	0.5	0	14	10	3.0	5	2.5	0	4.0
20	1.2	0	10	10	3.0	5	2.5	0	3.7
20	3.5	0	7.0	20	0.3	5	2.5	0	3.3
20	3.5	0	6.0	20	0.3	0	8.5	10	0.0
0	14.0	0	5.5	20	0.3	0	6.0	10	0.0
0	9.2	0	5.5	20	2.6	0	5.0	5	4.0
0	7.5	0	4.8	20	3.0	0	4.8	2.5	4.8
0	6.8	0	4.6	20	3.2	0	4.3	0	8.0
0	6.8	0	4.2	0	13	0	4.2	0	5.6
0	6.5	0	4.2	0	7.6	0	4.2	0	5.2
0	6.0	0	4.1	0	6.6	0	3.6	0	4.2
0	5.0	0	4.0	0	6.0	5	0.9	0	4.0
0	5.0	0	4.0	0	5.4	5	2.8	0	4.0
100	0.0	100	0.0	0	5.0	5	2.9	0	4.0
0	20	0	16	0	5.0	5	3.3	0	4.0

(The results of this experiment are plotted graphically in Fig. 3.)

1. That changes in the pressure deficit always have an immediate effect on the rate of absorption (Tables V–XI, Fig. 3).
2. The maximum effect usually follows immediately upon the change in pressure deficit (Table IX, and Fig. 3), but in some cases (e.g. Experiment 22 at 0 after exposure to 120 lb./in.) some little time may elapse before the maximum effect is reached.
3. An increase in pressure deficit invariably reduces, and a decrease in pressure deficit invariably increases the absorption rate irrespectively of

¹ See footnote¹, p. 230.

the previous history of the branch (Fig. 3), and that in general the greater the change in the pressure deficit the greater is the change in the absorption rate (Tables V–XI). This is shown by the figures given in Table XII, based on the results of Experiments 21 and 22.

TABLE X. (Experiment 22.)

Giving Mean Rates for Aesculus at 2-minute Intervals as in Experiment 21.

Press. Rate.	Press. Rate.	Press. Rate.	Press. Rate.	Press. Rate.	Press. Rate.
0	9.2	20	4.0	0	4.0
0	8.5	0	24	0	3.0
0	8.0	0	14	0	3.4
0	8.0	0	11	0	3.0
0	6.6	0	10	0	3.2
0	6.4	0	8.0	0	3.2
0	6.4	0	7.5	0	3.0
0	5.2	0	7.0	0	4.0
0	5.2	0	6.5	0	4.0
0	5.2	0	6.5	0	3.0
10	1.5	0	6.5	0	3.0
10	2.5	0	6.0	0	3.0
10	4.0	0	6.0	0	3.0
10	4.5	0	5.5	0	3.0
10	5.0	0	5.5	0	4.0
10	6.0	0	5.5	0	4.0
10	6.0	30	neg.	0	4.0
0	14.0	30	neg.	0	4.0
0	9.0	30	neg.	0	4.0
0	8.5	30	neg.	0	4.3
0	7.0	30	neg.	10	0.4
0	7.0	30	neg.	0	16
0	7.0	25	5.8	0	3.8
20	0.4	25	5.0	0	3.5
20	0.4	25	5.0	0	3.2
20	0.4	25	5.0	0	2.9
20	1.2	25	4.8	0	3.5
20	4.0	25	4.2	0	4.0
20	4.0	25	4.2	0	3.0
20	4.0	25	4.2	0	3.0
20	4.0	25	4.2	0	3.0
20	4.0	25	4.2	0	3.2

(Total length of experiment = 6 hrs. 12 mins.)

4. The reduced rate at an increased deficit slowly comes back towards the normal or original rate (Tables V, VIII, IX, X, XI), coming to a higher and more or less constant percentage of the original rate, which depends upon the increase (cf. Final values under different deficits in Table VIII, &c.).

5. The increased rate on release of a deficit falls off with time (Fig. 3) to a lower value depending upon the value of the deficit, and the other conditions of the previous history (Tables VI, IX, and X). The greater the deficit or the longer a given deficit is maintained, the greater is the increase in rate on return to 0 (see Fig. 3, and especially Tables VI and X).

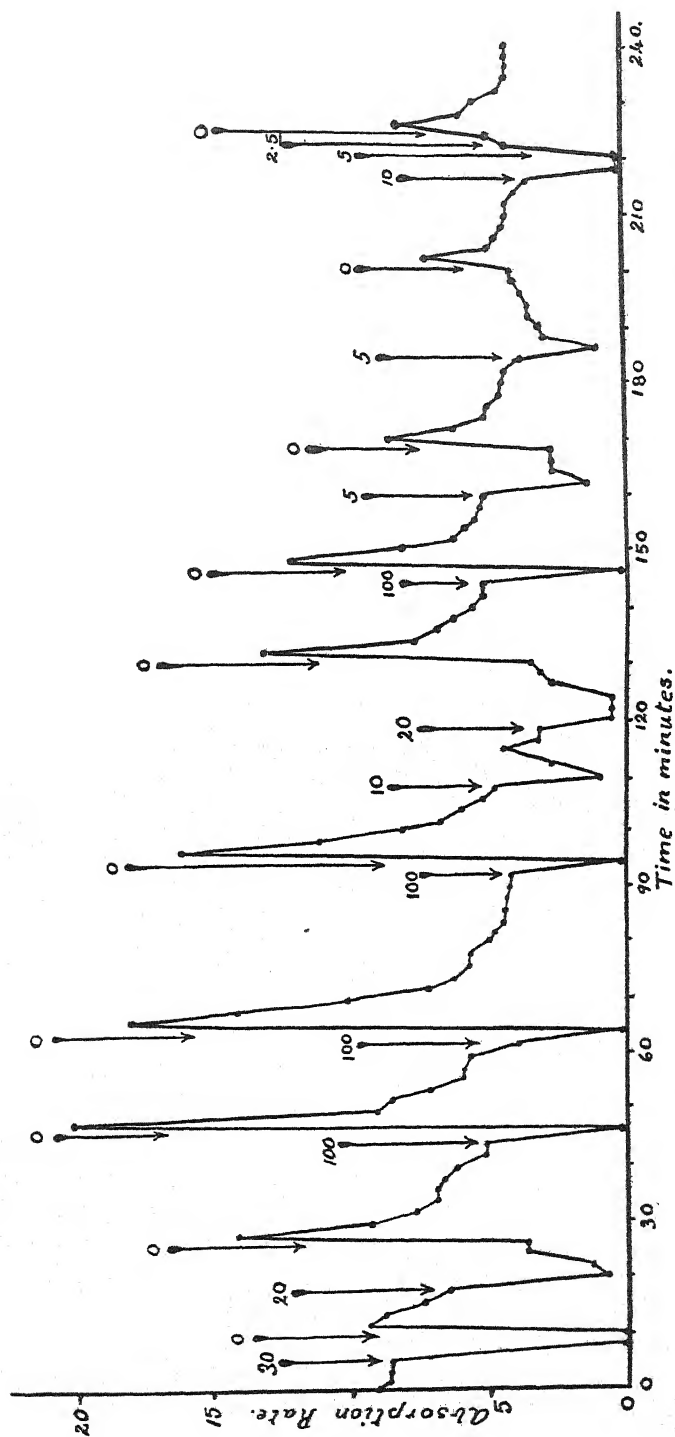


FIG. 3. Graph of results of Experiment 21, Table IX, showing changes in absorption rate on alterations of the pressure deficit. Absorption rate is plotted against time, the changes in the pressure applied being indicated by the arrows. The figures over the arrows give the pressures applied at these points in lb./in. (15 lb./in. = 1 atm.). For discussion see pp. 233, 234.

For instance, in Experiment 22, a deficit of 10 lb./in. maintained for 6 minutes is followed by an absorption rate at 0 of 3.3, while if maintained for 14 minutes it is followed by a rate of 10. A deficit of 20 lb./in. maintained for 10 minutes is followed by an absorption rate at 0 of 14, but if maintained for 18 minutes it is followed by a rate of 24 (Table X).

TABLE XI. (Experiment 23.)

Giving Mean Absorption Rates for Successive 2-minute Periods, Using Aesculus Under Various Pressure Deficits as in Experiments 21 and 22.

Press.	Rate.	Press.	Rate.	Press.	Rate.
0	8.0	15	5.0	0	3.0
0	9.0	0	8.0	0	3.0
0	8.0	0	7.0	0	2.8
0	7.0	0	5.0	0	2.8
0	6.0	0	5.0	0	2.8
35	0.0	0	4.0	0	2.6
10	8.0	35	0.0	20	1.1
10	5.0	0	3.6	20	1.2
10	5.0	10	2.5	0	4.5
10	5.0	10	3.5	0	4.0
0	9.0	10	4.5	0	3.6
0	7.0	0	7.0	40	neg.
0	7.0	0	5.5	0	8.3
0	6.0	0	4.5	0	7.7
0	5.0	0	4.5	0	7.7
0	5.0	0	4.0	0	5.9
15	3.0	0	3.5	0	4.2
15	4.0	0	3.5	0	3.8
15	5.0	0	3.0	0	3.6
15	5.0	0	3.0	0	3.6

TABLE XII.

In Experiment 21 the first change from

0 lb./in.	to	5 lb./in.	causes a decrease in rate to	25 per cent.
0	"	10	"	"
0	"	20	"	"
				16 per cent.
				8 per cent.

In Experiment 22, similarly the first change from

10 lb./in.	to	0 lb./in.	causes an increase in rate to	234 per cent.
20	"	0	"	"
25	"	0	"	"
				600 per cent.
				745 per cent.

6. The magnitude of the absorption rate at any given deficit depends in part upon the previous history of the branch (i.e. value and duration of other deficits experienced for some time past). Thus the rate at a pressure deficit of 0 (in Experiment 22) after a long period at 0 is 5.2. But after a deficit of 10 it is 14, after 20 it is 24, and after a period under a deficit of 25 lb./in. it is 28. Thus the greater the deficit the greater is the absorption rate at 0 when the deficit is first eliminated. Similarly the absorption at any given pressure deficit is considerably higher when the pressure is reduced to that value from a higher one, than when the pressure

is raised to that same value from a lower one. Thus in Experiment 22, at 25 lb./in. after a previous deficit of 30 lb./in. the absorption rate becomes 5.8, although before such a deficit had been applied the rate was only 5.5 at 0, and immediate increase of the pressure from 0 to 25 lb./in. reduced the absorption rate to 0 (not stated in the Table). As a further example, after subjection to 120 lb./in. (Experiment 22, Table X), the subsequent rate at 0 is over three times the rate at 0 before the high deficit was imposed. (Cf. also the rates at 0 before and after any increased deficit in any of the Tables V, VII, VIII, IX, and X.)

It also appears that the longer the period without changes in the pressure deficit the greater is the effect of a change of a given magnitude. (Cf. the different effects of exposure to 10 lb./in. after different lengths of 'rest period' at 0 in Experiment 22.) At the same time very high deficits and repeated changes in the deficit appear also to render the plant more susceptible to given changes in the deficit. (Cf. the different effects of the same change in pressure, e.g. from 0 to 10 lb./in., at different points in a long experiment, and especially before and after exposure to 120 lb./in. —Experiment 22.)

It is evident from these conclusions that consistent results defining the effect of any particular deficit will only be obtained by very uniform procedure on material that has not lately experienced any deficit, and that values of the absorption rate corresponding to different deficits must be studied independently when the deficits are varying, and when they are constant and sustained.

Experiments of these types will be recorded in a subsequent paper.

The full interpretation of the results of the present series in terms of changes in leaf-cell volume and transpiration rate will be evident from the conclusions reached on the basis of the later experiments.

SUMMARY.

The rates of absorption of water by a branch in a potometer have been followed when the upper parts of the branch are enclosed in a strong metal cylinder with a glass window for illumination and the leaves exposed to increased pressures.

The construction of the pressure cylinder is described.

Increases in pressure always decrease and decreases in pressure always increase the rate of absorption.

At sustained pressures above atmospheric the rate is at first reduced, but later recovers to some extent towards the original rate at atmospheric pressure. On reduction or release of the pressure there is an altogether disproportionate increase in the absorption rate, which however soon falls back towards the original level.

The actual rate of absorption at any pressure depends not only on the

pressure, but also upon the previous history of the branch in respect of lately experienced deficits.

Theoretical considerations show that the effects are probably in part due to alterations in leaf-cell volume, and in part due to alterations in transpiration rate. The experiments indicate the correct methods of procedure for determining the relative parts played by these two factors.

The parallelism with the effects of pressure deficits produced in nature is discussed.

The full interpretation of the results will be given later.

In conclusion, the writer wishes gratefully to acknowledge his indebtedness to Professor E. H. Lamb of the Engineering Department, Queen Mary College, for the valuable facilities offered for the frequent recharging of the supply cylinder, and also for the loan of a hand pump for the hydraulic tests; to Mr. F. C. L. Brendel for placing a Crossby Gauge Tester at the writer's disposal and for assistance in calibrating the Bourdon gauge; and to Mr. G. A. Wedgewood for checking the design of the pressure cylinder flanges. The cylinder and accessories were constructed in the College Instrument Shop, the expenses being defrayed by a grant from The Royal Society, to whom the writer is much indebted.

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On the Structure and Probable Affinities of Some Leaflets from Autun.

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With Plate VIII and two Figures in the Text.

IN the introductory part of a recent paper (11) reference was made to the occurrence of numerous small leaflets which were closely associated with the sterile and fertile leaflets of *Scolecopteris Oliveri* in siliceous nodules collected by Professor F. W. Oliver at Autun some years ago. A number of slides were prepared from this material by Mr. W. Heminway of Derby, some of which are in the author's collection whilst the remainder are in the private collection of the late Dr. D. H. Scott. In addition to this material I have been able, owing to the courtesy of Professor Paul Bertrand, to examine further specimens contained in slides in his father's collection (Slides 1193. 3, 1193. 4, 1193. 5) and also specimens present in three slides belonging to Professor Frère Carpentier of Lille. It is a pleasure to acknowledge the readiness with which these fellow palaeobotanists placed their slides at my disposal.

The leaflets were thought at first to be the sterile pinnules of the *Scolecopteris* (9), but a closer study revealed so many points of structural difference that it was realized that they must belong to another plant (11). In common with the other plant remains they frequently occur in more or less parallel layers, suggesting that they had fallen from nearby plants much as leaf mould occurs to-day and had been preserved *in situ*. The preservation is somewhat unequal, but in the best specimens is exquisite. In some examples, however, artefacts resulting from tissue alteration occur which are liable to misinterpretation.

Morphology.

The leaflets, which possessed strongly reflexed margins (Text-fig. 1, Pl. VIII, Figs. 1-3) were evidently lanceolate in shape with a midrib projecting prominently on the abaxial side. Measurements of a considerable number of sections give a maximum blade diameter of 2.3 mm., but the

diameter of the majority of the larger sections was round about 1.75 mm. The thickness of the leaflets in the region of the midrib was in most cases about 0.7 mm. and was relatively uniform. The following series of measurements taken from twenty approximately transverse sections will give some idea of the variation in dimensions:—

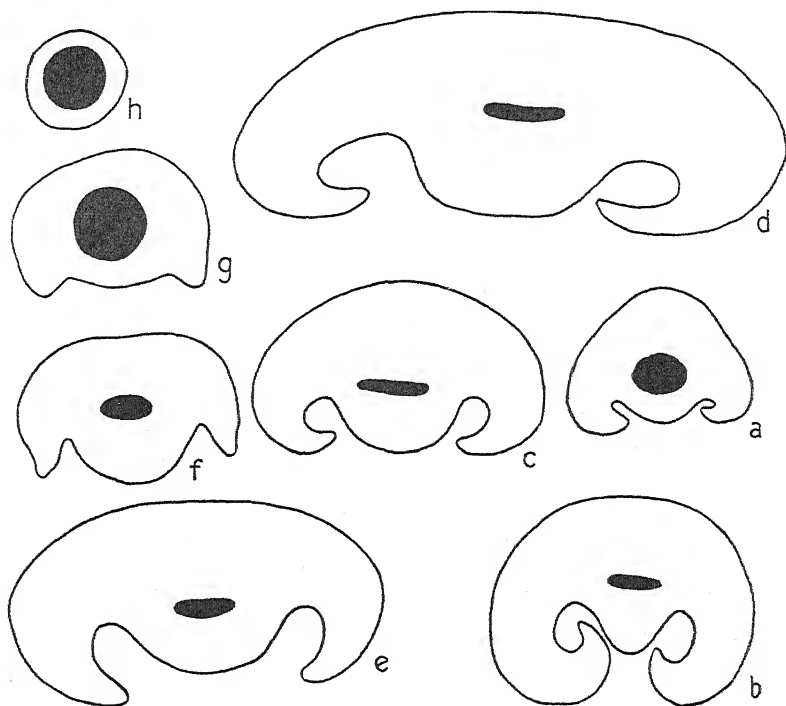
Diameter (mm.).	Thickness (mm.).	Diameter (mm.).	Thickness (mm.).
0.77	0.57	1.60	0.76
1.10	0.60	1.70	0.77
1.20	0.60	1.70	0.70
1.20	0.70	1.70	0.70
1.30	0.70	1.70	0.70
1.40	0.67	1.90	0.70
1.50	0.70	1.90	0.75
1.50	0.70	2.00	0.75
1.60	0.70	2.30	0.83
1.60	0.65	2.80	0.57

As will be seen below, there is reason to believe that the first two were sections cut near the apex whilst the last was cut near the base of the leaf. Some of the leaflets of which measurements were made are shown in outline in Text-fig. 1 in which the lettering is regarded as indicating the probable seriation from apex to base.

It will be obvious that Text-fig. 1 *a*, both from its dimensions and proportion of parts, is a section cut very near the apex of the leaflet, whilst the marked flexure of the margins and the size of the midrib in 1 *b* suggest that this section also is one cut near the distal end. Text-fig. 1 *d*, which illustrates the largest leaflet found, is probably one cut transversely through the middle of the blade, whilst the characters and measurements of 1 *c* and 1 *e* suggest that they would occupy positions above and below the middle respectively. The reduction in the amount of the lateral assimilatory parts of the lamina in 1 *f*, and its almost complete disappearance in 1 *g*, together with the change in the character of the vascular strand, render it probable that they represent sections of leaflets cut near the base.

The upper epidermis possesses an extremely thick cuticle and consists of elongated cells with dark contents (Pl. VIII, Figs. 2, 4). Over the greater part of the adaxial surface the long axis of the cells runs parallel to the midrib, but towards the reflexed margins of the leaflet this arrangement is replaced by one in which the long axis of the cells lies at right angles to the midrib. This is well shown in Pl. VIII, Fig. 4, which is an oblique section the plane of which passes through the epidermis in such a way as to cut it in the neighbourhood of the midrib on the right and near the margin of the leaflet on the left. The same feature is also illustrated in the transverse section of the leaflet shown in Pl. VIII, Fig. 2, where the contrast between the epidermal cells overlying the midrib and those at the edge of the leaflet is clearly marked.

The lower epidermis is essentially similar in character to that of the adaxial surface, but differs from it in possessing stomata which are distributed in the concavities lying on either side of the midrib. No really satisfactory examples of the stomata as seen in section have been

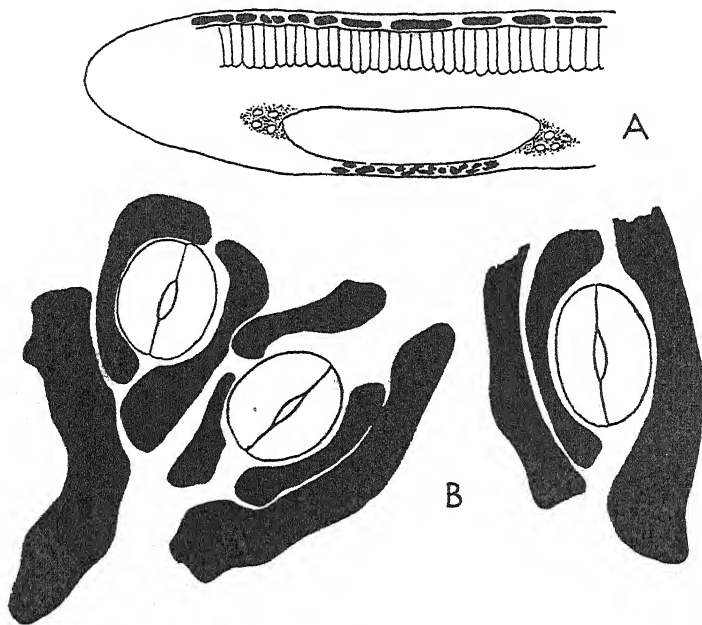


TEXT-FIG. 1. Camera-lucida outlines of a series of transverse sections of leaflets: the lettering suggests the probable seriation from apex to base ($\times 32$).

observed, but they are well shown in surface view in Slide No. 1193.3 from the Bertrand Collection and three of them are illustrated in Text-fig 2 B.

Situated immediately below the upper epidermis is a layer of well-developed palisade parenchyma interspersed with which are narrower cells, also elongated, possessing dark contents and presumably of a secretory nature (Pl. VIII, Figs. 1, 2). Below the palisade cells is a system of shorter cells which forms a relatively compact tissue radiating outward from the midrib. The latter is unbranched and is surrounded by a somewhat irregular sheath of large, longitudinally running, square-ended cells with dark contents (Pl. VIII, Figs. 5, 6). The vascular tissues consist of a tangentially flattened strip of xylem made up of small tracheids with annular or spiral thickenings below which is a similar strip of small-celled parenchyma which is considered to be phloem (Pl. VIII, Fig. 5). Lying on the abaxial side of the bundle is a well-defined system of large

parenchymatous cells which forms a prominent convexity below the conducting tissues. In some instances these cells appear to be thick-walled, but this appearance is only shown by specimens in which there is obvious evidence of decay and is almost certainly an artefact. There



TEXT-FIG. 2 A. Diagram of oblique section of leaflet indicating position of stomata ($\times 42$); 2 B. Three stomata from the same section ($\times 700$). (Coll. C. E. Bertrand, 1193-3.)

is some evidence that the midrib bundle expands near the apex so that it appears shortly ovoid in transverse section (Pl. VIII, Fig. 3). Unfortunately the preservation of the sections showing this feature leaves something to be desired, but it is probable that the modification indicates the presence of an apical 'water gland'.

Associated with the leaflets are a considerable number of small axes. The centre of each of these is occupied by a solid cylinder of xylem, which consists of tracheids with spiral and annular thickenings, these showing a remarkable resemblance to the xylem elements found in the Psilophytales. Apart from their larger calibre they also resemble the tracheids constituting the xylem of the leaflet midribs (Pl. VIII, Figs. 9, 10). No clearly defined protoxylem was seen. The tracheids are enclosed in a thin sheath of phloem, and outside this a single layer of relatively large parenchymatous cells with dark contents may be detected in some examples. Beyond this is a narrow parenchymatous cortex two or three cells in width, this being succeeded by an epidermis consisting of

cells possessing a thick cuticle and dark contents. The epidermis thus resembles that of the leaflets, and this resemblance is emphasized by their shape as seen in surface view. A comparison of the obliquely tangential section of the axis illustrated in Pl. VIII, Fig. 11, in which the epidermal cells are shown in surface view with Figs. 2 and 4, demonstrates this very clearly.

There are thus some grounds for believing that axes and leaflets were parts of the same plant. It remained, however, to determine the exact nature of the axes themselves as well as to try to find whether any further evidence was available which supported the view indicated above as to their relationship to the leaflets.

With regard to their nature, the thick cuticle and the bulk of the stele relative to the axis as a whole renders it unlikely that they were roots. The choice thus lies between their being stems or petioles. Against their being considered stems is the fact that in not one example of the many specimens examined is there any indication of either branching or the giving off of traces to lateral members, whilst such positive evidence as there is lends definite support to their being regarded as petioles. The first discovery consisted of a transverse section in which the stele still obviously possessed a cylindrical core of tracheids surrounded by a parenchymatous cortex of rounded cells, although the axis itself was ovoid with a small but definite flange on either margin (Pl. VIII, Fig. 8). The preservation is unfortunately of the 'shadowy' type which is not infrequent in siliceous nodules, but it is sufficiently good to render the essential characteristics clear. A second example, also ovoid, whilst showing a stele of similar type was characterized by the fact that definite palisade tissue is developed in the cortex, whilst yet a third, a camera-lucida outline of which is shown in Text-fig. 1*f*, has a definite flattening of the bundle with a well-developed palisade tissue and a definite though restricted lamina on either side. There is thus a transitional sequence between the type of structure characterizing the axis and that characterizing the leaflet.

DISCUSSION.

It is evident that the leaflets described in the preceding part of this paper were the foliar members of a plant of xerophytic type, as indicated by the thick cuticle, the strongly recurved margins, and the restriction of the stomata to the under side. Judged by the remains of other plants accompanying it the parent plant appears to have been a constituent of a flora subjected to drought conditions, *Scolecopteris Oliveri*, for example, also having recurved leaflets (11). In *Scolecopteris* the cuticle was thin, but special water-storing mesophyll gave the leaf a fleshy character, whilst the less complete remains of other plants, some of which are probably

pteridosperm pinnules, also show epidermal modifications for the protection of the stomata. The available data suggest that the plant grew under arid or semi-arid conditions, and this rather militates against the suggestion made by Carpentier (2, 3) that the dark contents of the epidermal cells are due to assimilatory products. Portions of leaflets obviously identical with the ones described here were figured by Renault as long ago as 1878 (7), and the same author also figures a fairly well-preserved transverse section in his classic 'Flore fossile d'Autun et d'Epinac' (8). Renault considered that they were probably prolongations of the fibrous bases of the sterile bracts of *Bruckmannia* (*Calamostachys*) *Grand'Euryi*. They were evidently often associated with calamitean cones in his material, and a similar association is to be noted with the cone described by Browne (1) in 1925 as *Calamostachys Grand'Euryi* f. *Decaisnei*, a readily recognizable section being present in Plate XI, Fig. 16, of her paper. It is quite evident that these were true foliar members and not prolongations of cone bracts as, where the latter are adequately known, they are much more strongly sclerosed and contain very little assimilatory mesophyll.

Professor Carpentier in a general study of material from Grand Croix has also shortly described the leaflets of our plant and, as stated at the beginning of this paper, has generously handed over to me for study the three slides upon which his description is based (3). Carpentier considers that the leaflets belonged to some species of *Annularia*, an attribution which is supported by their lanceolate shape, by the possession of a simple, unbranched midrib, and, possibly, by the presence of a bundle sheath of melasmatic cells. He also notes the fact that the impression species *Annularia stellata* has leaflets of similar dimensions possessing reflexed margins. Species of *Annularia* showing leaflets with reflexed margins are also known from the British Coal Measures as impressions, and I am greatly indebted to Professor Walton of Glasgow for giving me an opportunity of examining slides of some of these.

Whilst, however, the known facts suggest the attribution of the leaflets to some calamitean plant, it should be realized that the guard-cells of the stomata do not show the lignified bars which are a remarkably constant feature in both palaeozoic, mesozoic, and recent Equisetales. We have, moreover, no evidence whatever as to the nature of the stems upon which the leaflets were borne nor of their arrangement. Until this information is available we must regard their assignation to *Annularia* as purely tentative.

Isolated leaflets of an essentially similar character to those present in the French silicified material have been described by Hoskins (5) from the Pennsylvanian of Illinois. Hoskins states that these are 'undoubtedly calamitean' without, however, giving details of the evidence upon which this view is based. There are minor but obvious specific differences

between the American species and that from France, of which the following may be noted:—

1. The greater diameter of the leaflets (Hoskins gives this as 2.6 mm.).
2. The less pronounced flexure of the margins.
3. The difference in the structure of the midrib which is 'composed of a circular group of specially thickened tracheids . . . of very small caliber' which 'do not always constitute a solid strand, but are sometimes separated by cells with unthickened walls'.
4. The much more evident development of spongy mesophyll.

The occurrence of these leaflets in American coal balls provides a further instance of the affinities between the Pennsylvanian flora as represented in petrified material and that of the Stephanian of France. Hoskins has already recorded the presence of a *Scolecopteris* (*S. minor*) (4) comparable with the French *S. Oliveri* and of a *Botryopteris* closely resembling *B. forensis* (6), and it seems probable that further resemblances will be discovered as the Pennsylvanian flora is more intensively studied.

SUMMARY.

An account is given of the morphology of some isolated leaflets from silicified material collected at Autun. These are characterized by their downwardly flexed margins and their lanceolate shape. Each possesses a thick cuticle and an epidermis of elongated spindle-shaped cells with dark contents.

Stomata are present and are confined to the abaxial surface of the leaflet where they are grouped in the concavities on either side of the midrib.

Well-developed palisade tissue is present interspersed with narrower cells of similar shape having dark contents.

The midrib consists of a tangentially flattened strip of small tracheids and is surrounded by a sheath of melasmatic cells. It is unbranched.

There is evidence that the leaflets possessed a short petiole traversed by a cylindrical core of spiral and annular tracheids.

The anatomy suggests calamitean affinities, but the absence of any evidence as to the nature of the axis upon which they were borne renders definite assignation to this group impossible.

The author is greatly indebted to the late Dr. D. H. Scott both for the loan of slides and for his kindly interest in the investigation.

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EXPLANATION OF PLATE VIII.

Illustrating Professor H. S. Holden's paper on 'The Structure and Probable Affinities of Some Leaflets from Autun'.

All the figures are from untouched negatives.

Fig. 1. Transverse section of leaflet showing distribution and character of tissues (148.B5, Author's Coll.) $\times 40$.

Fig. 2. Part of a transverse section of a leaflet showing the thick cuticle and dark contents of the epidermal cells. Note the melasmatic cells round the midrib and the cells with dark contents among the palisade cells (148.7, Scott Coll.) $\times 60$.

Fig. 3. Transverse section of a leaflet cut near the tip. Note the increase in dimensions of the midrib bundle (148.B3, Author's Coll.) $\times 70$.

Fig. 4. Oblique surface section showing to the right the epidermal cells in the neighbourhood of the midrib and to the left the epidermal cells at the margin of the leaflet (Carpentier Coll.) $\times 40$.

Fig. 5. Transverse section of the midrib and adjacent tissues to show details of xylem and surrounding parts (148.11, Author's Coll.) $\times 90$.

Fig. 6. Part of leaflet in longitudinal section showing the bundle sheath (148.11, Author's Coll.) $\times 60$.

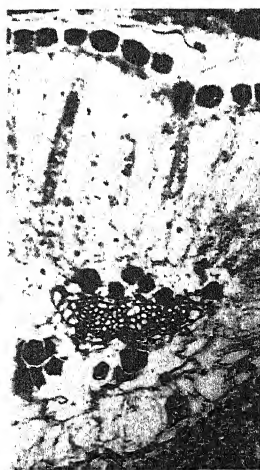
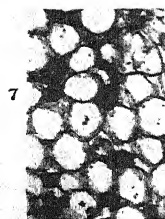
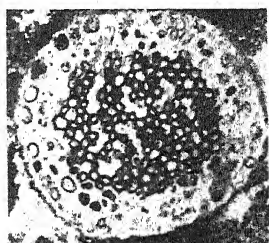
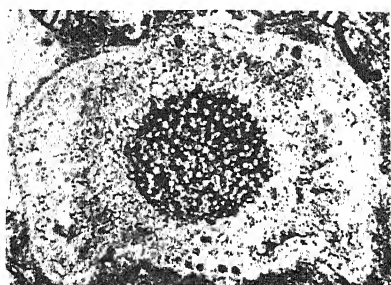
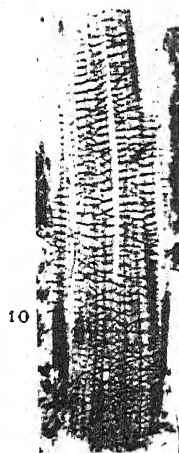
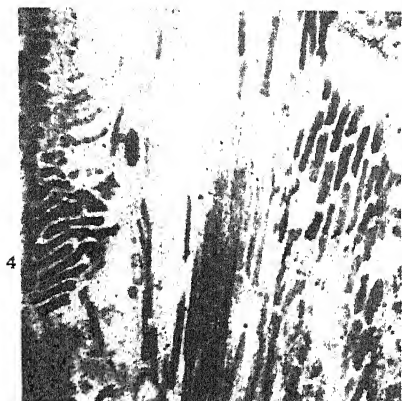
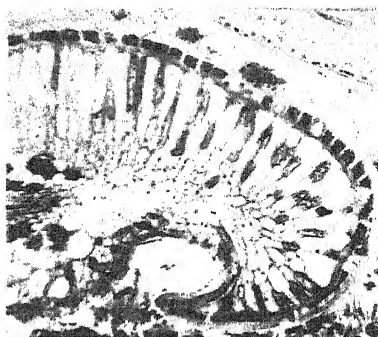
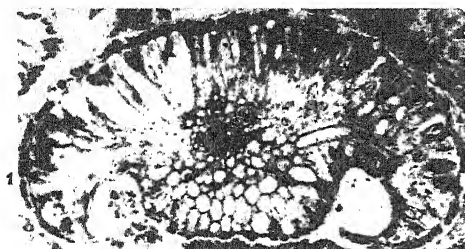
Fig. 7. Small part of a section through the palisade parenchyma parallel to the surface of the leaflet. Note the dark cells among the palisade cells (148.B6, Author's Coll.) $\times 90$.

Fig. 8. Transitional section through the transitional region between the petiole and leaflet. Note the marginal flanges, the cylindrical midrib, and occasional epidermal cells with dark contents (148.B3, Author's Coll.) $\times 60$.

Fig. 9. Transverse section of petiole (148.B2, Author's Coll.) $\times 80$.

Fig. 10. Longitudinal section of xylem of petiole (148.B5, Author's Coll.) $\times 90$.

Fig. 11. Oblique section of petiole. Compare the epidermal cells with those of the leaflet shown in Fig. 4 (148.B4, Author's Coll.) $\times 90$.



Translocation and Growth Balance in Woody Plants.¹

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With twelve Figures in the Text.

CONTENTS

	PAGE
INTRODUCTION	247
MATERIALS AND METHODS	248
THE TRANSLOCATION OF FOOD MATERIALS IN WOODY PLANTS	249
The disappearance of stored starch	249
The volume-weight of apple wood	250
Twig and leaf development on ringed branches	250
The utilization of stored food in two-year-old poplar, and the effect of ringing	253
THE BALANCE OF APICAL AND DIAMETER GROWTH	261
The effect of ringing upon diameter growth	261
The effect of ringing upon apical growth	262
Growth 'hormones'	265
Chemical composition and growth balance	265
Translocation and polarity	269
DISCUSSION AND SUMMARY	269
LITERATURE CITED	271

INTRODUCTION.

FOOD stored in the stems of woody perennials is commonly assumed to be translocated upward for the initiation of shoot and leaf growth in the spring. Hartig (11) decided that this upward translocation occurred in the xylem, and his findings were widely accepted until the recent work of Curtis (3, 5, 7) and Mason and Maskell (17, 18, 19), which appears to have established the phloem as the normal channel of upward as well as of downward translocation of organic foods. In addition, Curtis (4) has shown that upward translocation is limited in both time and extent, and that a downward movement of foods starts as soon as photosynthesis becomes active. Hastings (12), Knudson (13), and others have found that

¹ This study was made possible by a grant from the Rockefeller Fluid Research Fund.

cambial growth in trees is not initiated until the leaves are well advanced, and presumably after this downward translocation has begun, and Proebsting (21) has shown that secondary growth can be interrupted by defoliation. Both upward translocation and secondary growth would thus seem to be limited as outlets for the stored food of tree trunks.

The experiments reported here have been concerned with the fate of the stored and current foods; with the accompanying problems of the channels, direction, and substances of translocation at various periods in the seasonal development of woody plants; and with the factors affecting the balance of primary and secondary growth.

MATERIALS AND METHODS.

Four lots of trees have been used for these experiments. One hundred and twenty five-year-old Wealthy apple trees (*Pyrus malus* L.) were used in 1929; 165 two-year-old poplar whips (*Populus nigra* L.) were used in 1930; 17 box elder seedlings (*Acer Negundo* L.) averaging about twelve years old, and 50 framework branches on 10 fifteen-year-old apple trees made up the material for 1932. Ringing and other treatments were replicated at least five and usually ten times, and samples for chemical analyses were composed of material from an equal number of individuals.

Cambial growth was measured as circumference with a steel tape and recorded as a percentage of the winter measurement. Five paint spots on each trunk segment indicated the points to be measured, and with ten replications each growth record shown in the data is the average of fifty measurements.

Chemical samples were composites of from five to ten tree segments. Wood and bark were separated and cut rapidly into boiling 95 per cent. alcohol. The samples were extracted by decantation with 80 per cent. alcohol. Sugar samples were cleared with neutral lead acetate and delead with sodium oxalate. Sucrose was hydrolysed with invertase; starch was brought into solution with taka-diaxase, and hydrolysis completed with acid after neutral-lead clearing. Reducing sugars were determined with a modified Munson Walker-Bertrand technique and calculated as glucose or invert sugar. Nitrogenous materials extracted by 80 per cent. alcohol were determined as soluble or non-colloidal nitrogen. This fraction was composed almost entirely of organic nitrogen, largely amino and basic or ureid compounds. Nitrogen not extracted was designated as insoluble or colloidal, and considered to be an indication of the protein nitrogen of the sample.

Ammonia was determined from the alcohol extract by the Folin aeration method, and amide nitrogen by the same method after acid hydrolysis. Nitrates and nitrites were checked with devarda alloy from the residue of the amide determination. Amino nitrogen was estimated by the

Van Slyke method, and basic nitrogen was precipitated with phosphotungstic acid.

THE TRANSLOCATION OF FOOD MATERIALS IN WOODY PLANTS.

Rings which removed the phloem with minimum injury to the xylem were used to control food movement in these experiments, and to bring about varying nutrient conditions within the trunk segments under observation. The exposed xylem was protected with paraffin, and reunion of the phloem by callus growth was prevented.

The Disappearance of Stored Starch.

One hundred and twenty five-year-old Wealthy apple trees were used in 1929 for growth measurements, including the effect of ringing at monthly intervals through the summer on growth and sprouting (Fig. 9). All branches were removed to a height of 24 in. and the trunk was marked into 'base', 'middle', and 'top' segments. Ringing treatments consisted of two phloem rings which separated the three segments. Lots of ten check trees and ten trees ringed on April 7 were dug at monthly intervals for observation of food reserves. At the time of taking the samples, representative tissues were sectioned and observed for starch storage. On April 7 growth had not started, and the winter condition of well-filled cortex and wood rays throughout the plant and of well-filled pith in the one-year twigs and xylem in the roots was observed.

On May 6 new growth was one-half inch long and the first leaves one-fourth grown. While there was some evidence that more starch had been removed from the upper portions of the plants, the most striking change was the removal of starch from the cortex of both tops and roots with the progressive depletion of the wood rays and parenchyma. Pith stores were practically unaffected. Plants ringed at two levels on the trunk on April 7 showed on May 6 about the same loss of starch from the upper portions of the tree as did the checks; more starch was left between the trunk rings and much more in the roots of the ringed trees. These data might indicate that in trees of this size there is normally some upward translocation from the roots, and that the rings had interfered with this movement.

On July 9 new growth of the small apple trees averaged 15.8 in. on the check and only 6.7 in. on the girdled trees, possibly further evidence of interference with upward translocation. Observations, however, showed that there was less starch between the trunk rings than in the corresponding segment of the checks, and much less starch in the roots of the ringed plants. Starch had accumulated markedly in all parts above the rings, including the new growth, but only slightly in the upper portions of the checks. The disappearance of starch, from both the roots and between

the rings, corresponds to the observations of Hartig (11), which he considered to be evidence of upward translocation in the transpiration stream. Attempts to eliminate the transpiration stream by removing all branches failed because of the sprouting of the base and death of the central and upper sections of the trunk. Data to be presented later show that the food from between the rings or below them was not used in diameter growth of the trunk.

The Volume-Weight of Apple Wood.

The trunk segments used for starch observation were measured for volume by displacement of alcohol, and dried. The samples were then ground, and an attempt was made to measure their reserve food by loss in weight with acid hydrolysis. The residues from the hydrolysis varied with slight variations in hydrolysing technique, and were found to form a poorer index of reserves than the dry matter of a cubic centimetre of green trunk section. These data for the roots, middle, and top of ringed and check plants are plotted in Fig. 1. The delayed loss of dry matter from between the rings and an accumulation above the rings after photosynthesis started are clearly shown. The rapid loss of dry matter from between the rings during May and June strongly suggests leakage across the rings, an hypothesis which is supported by chemical analyses to be reported later. As explained above, no measurable diameter growth was observed below the top ring, and the few sprouts which developed on this material were removed weekly before they had progressed beyond the bud stage, so that only respiration and leakage are available as explanations of the observed loss. The rapid disappearance of dry matter from the roots of both the check and the ringed plants without even a temporary check in rate of loss as the result of ringing, as contrasted with the starch observations, indicates that materials other than starch were being used, and suggests that stored foods can be used in new root growth and that the bulk of the food stored in the roots of these trees was normally so used. The initiation of root elongation before bud growth starts has been commonly observed, but, as far as the author is aware, no studies on the utilization of stored food in the secondary growth of roots have been published.

Twig and Leaf Development on Ringed Branches.

We have indicated that sprout growth was retarded by ringing five-year-old apple trees. The early top growth of two-year-old poplar whips was also reduced by trunk rings, and the leaves of the ringed trees were injured by a late frost which did not affect the untreated plants. Dexter (8) has shown that frost hardiness depends upon an adequate food supply in

the tissues, and the observations suggest that at least a temporary upward movement of food from the trunk and roots occurs in these small plants. On the other hand, the early growth of ringed, six-year-old poplar trees

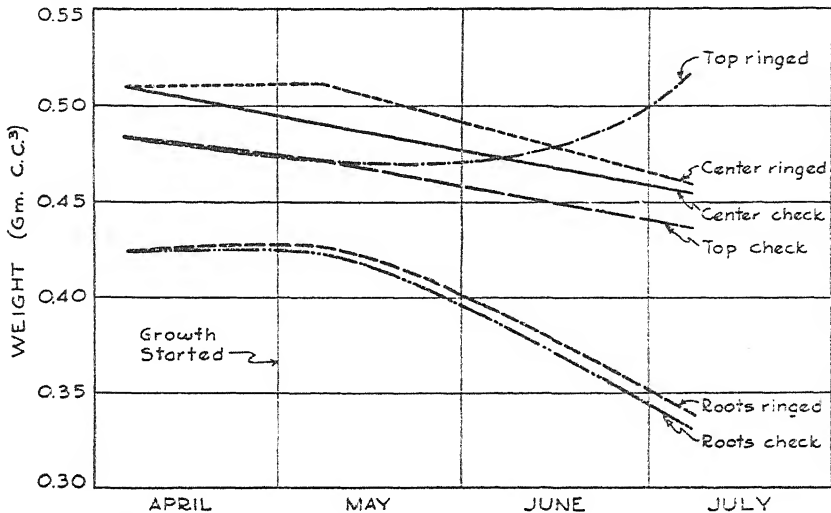


FIG. 1. The effect of growth and ringing upon the volume-weight of apple wood. 1929 experiments. 'Top ringed' was the trunk section immediately above the rings, 'middle ringed' was between, and roots of course below the rings. Checks were corresponding areas of unringed trees. All data are the average of individual measurements on ten tree segments.

was indistinguishable from that of the checks. The larger trees had a greater and apparently adequate storage area in the branches and upper trunk.

A study of the effects of ringing the main branches of fifteen-year-old apple trees upon apical growth, diameter growth, leaf area, and composition, throws light upon the effect of phloem rings on the movement of food materials in this plant. Matched pairs of limbs on each of five trees were chosen for the experiment; one limb of each pair was ringed and the other used as check. Growth and leaf composition data taken in September after ringing in April are shown in Fig. 2. These limbs were 2-2½ in. in diameter at the point of ringing, and the ringed limbs produced an essentially normal shoot, diameter and leaf growth in the first season. The leaves of the ringed limbs also contained a normal percentage of ash, particularly on an area basis, but were significantly lower in total nitrogen than the checks. The 97 per cent. sprout growth made by the apical buds of the ringed limbs is somewhat misleading since apical growth on these branches was confined to a smaller number of buds which individually, made normal growth, presumably upon nitrogenous food materials stored within the branch (Fig. 8). In the second season a very decided effect of ringing upon growth was observed. Diameter growth of the ringed limbs

was only 43 per cent. of the check as compared to 108 per cent. in the first season, and apical growth dropped from 97 to 7 per cent. of the check. The leaves of the ringed branches fell prematurely the second season, and

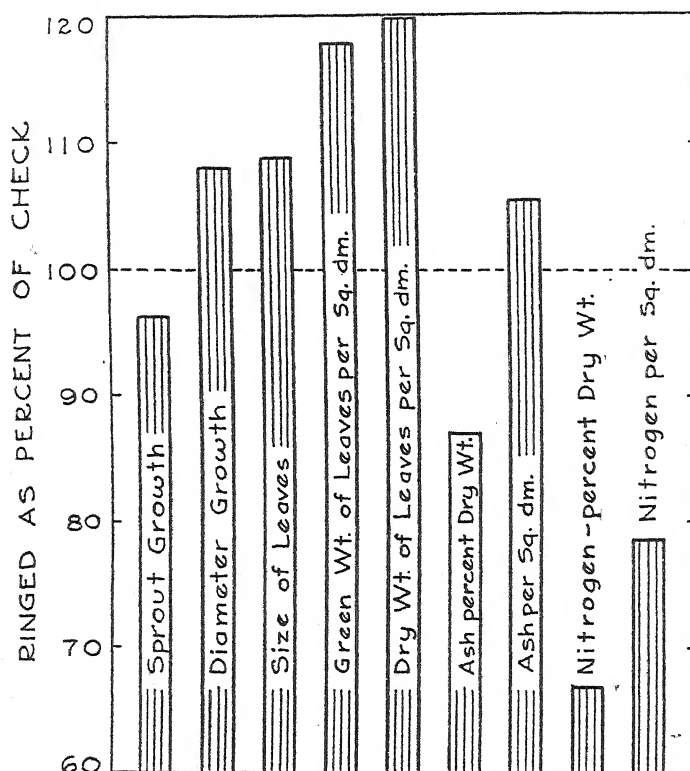


FIG. 2. Growth and composition of large ringed branches in per cent. of normal. All data are for the first season after ringing. Results for the second season are given in the text.

no leaf samples were obtained. New twig growth on the ringed branches was too scant to provide macro-samples, but analyses of the bark and wood showed only two-thirds as much nitrogen in the bark of the ringed branches as was present in the checks.

The observations of Curtis (4) that only the twigs and medium-sized branches are drawn upon for stored food to start spring growth would appear to be applicable to our results, with the reservation that the trunk and roots may be drawn upon by young trees or shrubs having a relatively small aerial storage space. The nitrogen deficiencies in the ringed branches will be considered below with other data indicating that the normal upward movement of nitrogen in the plants studied occurs in the phloem.

The Utilization of Stored Food in Two-year-old Poplar and the Effect of Ringing.

Analyses of the bark and wood of two-year-old poplars (*Populus nigra* L.) used in the 1930 experiments, supply information regarding the utilization of stored food in plants of this size. Samples were taken on April 28, just before the buds opened and at a time when the chemical conditions of normal sprouting should be demonstrated; on May 23 when the new leaves were well grown and cambial growth was beginning, and on June 16, six weeks after growth started. The trunks of these trees were pruned to a height of 30 in. unless otherwise noted and were marked off into 'base', 'middle', and 'top' segments, as described for the apple, with the rings of ringed trees separating the three segments and leaving the 'base' connected to the roots and the 'top' to the upper part of the plant, but the 'middle' segment isolated so far as phloem connexions were concerned. All rings were cut on April 28. A ten-tree sample collected on April 28 served as the base for both check and ringed comparisons. Ten trees each of check and ringed were cut on the other two dates; the segments were separated into bark and wood, and analysed according to the outline previously given. The composition of the wood and bark samples on the various dates is given in Tables I and II, and the trends of three nitrogen and three carbohydrate fractions in the bark are presented in Figs. 3-8.

TABLE I.
Composition of the Bark of Poplar Tree Segments.

Trunk segment.	Chemical fraction.	Mg. in 100 gm. of fresh sample on		
		April 28.	May 23.	June 16.
Base of check (unringed)	Reducing sugars	1359.5	986.0	837.0
	Sucrose	1579.2	814.0	670.5
	Total sugars	2938.7	1800.0	1507.5
	Starch	3060.0	1889.0	2049.0
	Total carbohydrates	5998.7	3689.0	3556.5
	Protein nitrogen	395.0	177.3	167.0
	Soluble nitrogen	58.7	60.7	40.7
	Total nitrogen	453.7	238.0	207.7
Middle of checks (unringed)	Reducing sugars	1319.5	964.7	756.0
	Sucrose	1421.2	938.8	756.0
	Total sugars	2740.7	1903.5	1512.0
	Starch	3563.0	2077.0	2186.0
	Total carbohydrates	6303.7	3980.5	3698.0
	Protein nitrogen	442.3	237.8	193.1
	Soluble nitrogen	74.7	60.3	58.7
	Total nitrogen	517.0	298.1	251.8

TABLE I (continued).

Trunk segment.	Chemical fraction.	Mg. in 100 gm. of fresh sample on		
		April 28.	May 23.	June 16.
Top of checks (unringed)	Reducing sugars	1070.4	945.5	754.0
	Sucrose	1287.6	888.0	767.5
	Total sugars	2358.0	1833.5	1521.5
	Starch	3505.5	2207.0	1986.0
	Total carbohydrates	5863.5	4040.5	3507.5
	Protein nitrogen	467.4	242.4	204.8
	Soluble nitrogen	65.3	73.2	66.0
	Total nitrogen	532.7	315.6	270.8
Base of ringed (below rings)	Reducing sugars	1359.5	792.5	543.0
	Sucrose	1579.2	841.5	409.7
	Total sugars	2938.7	1634.0	952.7
	Starch	3060.0	2104.0	2523.0
	Total carbohydrates	5998.7	3738.0	3475.7
	Protein nitrogen	395.0	411.5	304.2
	Soluble nitrogen	58.7	161.3	169.3
	Total nitrogen	453.7	572.8	473.5
Middle of ringed (between rings)	Reducing sugars	1319.5	863.0	538.0
	Sucrose	1421.2	1204.0	802.0
	Total sugars	2740.7	2067.0	1340.0
	Starch	3563.0	2981.0	3924.0
	Total carbohydrates	6303.7	5048.0	5264.0
	Protein nitrogen	442.3	415.0	247.3
	Soluble nitrogen	74.7	155.4	77.3
	Total nitrogen	517.0	570.4	324.6
Top of ringed (above rings)	Reducing sugars	1070.4	953.0	1197.5
	Sucrose	1287.6	514.5	1301.5
	Total sugars	2358.0	1467.5	2499.0
	Starch	3505.5	1947.0	5135.0
	Total carbohydrates	5863.5	3414.5	7634.0
	Protein nitrogen	467.4	257.5	217.8
	Soluble nitrogen	65.3	76.0	46.1
	Total nitrogen	532.7	333.5	263.9
Middle of ringed with leaves (between rings with full foliage allowed to de- velop)	Reducing sugars	1319.5	1000.0	1148.5
	Sucrose	1421.2	408.0	1479.5
	Total sugars	2740.7	1408.0	2628.0
	Starch	3563.0	2133.0	6217.0
	Total carbohydrates	6303.7	3541.0	8845.0
	Protein nitrogen	442.3	170.2	162.7
	Soluble nitrogen	74.7	95.3	36.4
	Total nitrogen	517.0	265.5	199.1

TABLE I (*continued*).

Trunk segment.	Chemical fraction.	Mg. in 100 gm. of fresh sample on		
		April 28.	May 23.	June 16.
Middle of ringed defoliated (between rings with all buds growing but leaves removed)	Reducing sugars	1319.5	—	985.0
	Sucrose	1421.2	—	269.5
	Total sugars	2740.7	—	1254.5
	Starch	3563.0	—	2507.0
	Total carbohydrates	6303.7	—	3761.5
	Protein nitrogen	442.3	—	167.5
	Soluble nitrogen	74.7	—	57.9
	Total nitrogen	517.0	—	225.4

TABLE II.

Composition of the Wood of Poplar Tree Segments.

Trunk segment.	Chemical fraction.	Mg. in 100 gm. of fresh sample on		
		April 28.	May 23.	June 16.
Base of checks (unringed)	Reducing sugars	107.3	140.0	236.5
	Sucrose	381.0	259.0	174.0
	Total sugars	488.3	399.0	410.5
	Starch	3272.5	2476.0	2688.0
	Total carbohydrates	3760.8	2875.0	3098.5
	Protein nitrogen	72.4	80.8	160.2
	Soluble nitrogen	40.6	9.7	2.6
	Total nitrogen	113.0	90.5	162.8
Middle of checks (unringed)	Reducing sugars	136.2	172.7	337.5
	Sucrose	496.5	399.3	183.2
	Total sugars	632.7	572.0	520.7
	Starch	3011.5	2277.0	2336.0
	Total carbohydrates	3644.2	2849.0	2856.7
	Protein nitrogen	90.3	104.6	90.2
	Soluble nitrogen	35.1	13.4	9.6
	Total nitrogen	125.4	118.0	99.8
Top of checks	Reducing sugars	224.0	259.0	402.5
	Sucrose	510.5	290.5	76.0
	Total sugars	734.5	549.5	478.5
	Starch	2726.0	2243.0	1916.0
	Total carbohydrates	3460.5	2792.5	2394.5
	Protein nitrogen	123.9	122.8	64.2
	Soluble nitrogen	27.2	17.0	19.0
	Total nitrogen	151.1	139.8	83.2

TABLE II (*continued*).

Trunk segment.	Chemical fraction.	Mg. in 100 gm. of fresh sample on		
		April 28.	May 23.	June 16.
Base of ringed (below rings)	Reducing sugars	107.3	115.0	0.0
	Sucrose	381.0	170.2	25.6
	Total sugars	488.3	285.2	25.6
	Starch	3272.5	2358.0	1763.0
	Total carbohydrates	3760.8	2643.2	1788.6
	Protein nitrogen	72.4	85.7	49.0
	Soluble nitrogen	40.6	19.0	49.6
	Total nitrogen	113.0	104.7	98.6
Middle of ringed (between rings)	Reducing sugars	136.2	130.0	135.0
	Sucrose	496.5	351.0	153.8
	Total sugars	632.7	481.0	288.8
	Starch	3011.5	2246.0	2194.0
	Total carbohydrates	3644.2	2727.0	2482.8
	Protein nitrogen	90.3	107.2	63.1
	Soluble nitrogen	35.1	28.6	45.7
	Total nitrogen	125.4	135.8	108.8
Top of ringed (above rings)	Reducing sugars	224.0	109.7	384.0
	Sucrose	510.5	138.3	364.5
	Total sugars	734.5	248.0	748.5
	Starch	2726.0	2713.0	2392.0
	Total carbohydrates	3460.5	2961.0	3140.5
	Protein nitrogen	123.9	86.8	62.6
	Soluble nitrogen	27.2	17.4	17.2
	Total nitrogen	151.1	104.2	79.8
Middle of ringed leaves (between rings with full foliage allowed to de- velop)	Reducing sugars	136.2	150.0	352.7
	Sucrose	496.5	65.0	428.9
	Total sugars	632.7	215.0	781.6
	Starch	3011.5	2050.0	3246.0
	Total carbohydrates	3644.2	2265.0	4027.6
	Protein nitrogen	90.3	52.6	61.9
	Soluble nitrogen	35.1	19.6	14.5
	Total nitrogen	125.4	72.2	76.4

The data show a heavy drain on both carbohydrates and protein (insoluble) nitrogen during early shoot growth and extending into June. More than 40 per cent. of the total carbohydrates and 56 per cent. of the proteins of the bark of the trunk were used between April 28 and June 16.

The wood lost somewhat less carbohydrate and considerably less nitrogenous material. In fact the base segment was gaining insoluble nitrogen on June 16, suggesting either re-storage or the effect of new cell production.

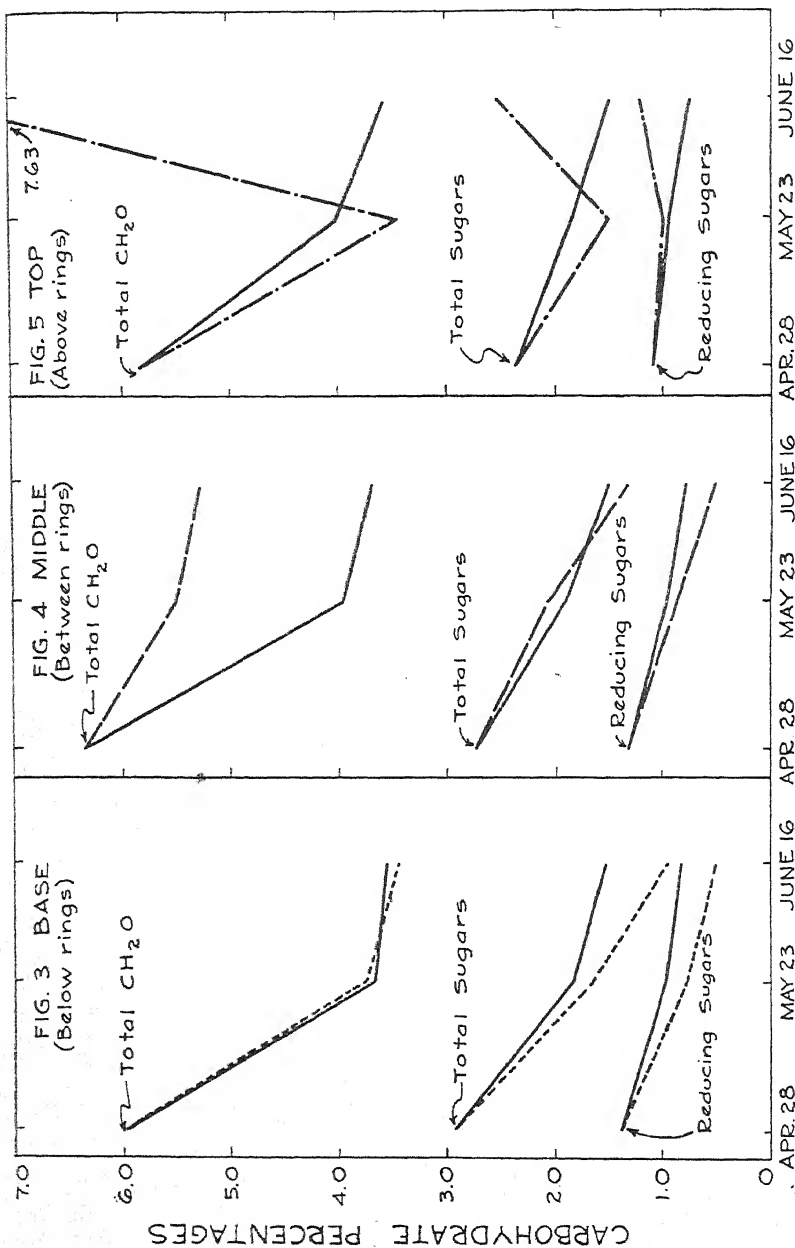
The effect of phloem rings is evident, particularly in the figures, where check and ringed segments are plotted together. The rapid loss of carbohydrates from below the rings (Fig. 3); the early loss from above the rings and the later gain (Fig. 5) and the loss of carbohydrates from between the rings, shown in Fig. 4, correspond to the 1929 results shown in Fig. 1. The loss of carbohydrates from between the rings might be explained as leakage, but could perhaps have been due to respiration with some callus and sprout growth in the isolated, between-rings segment.

TABLE III

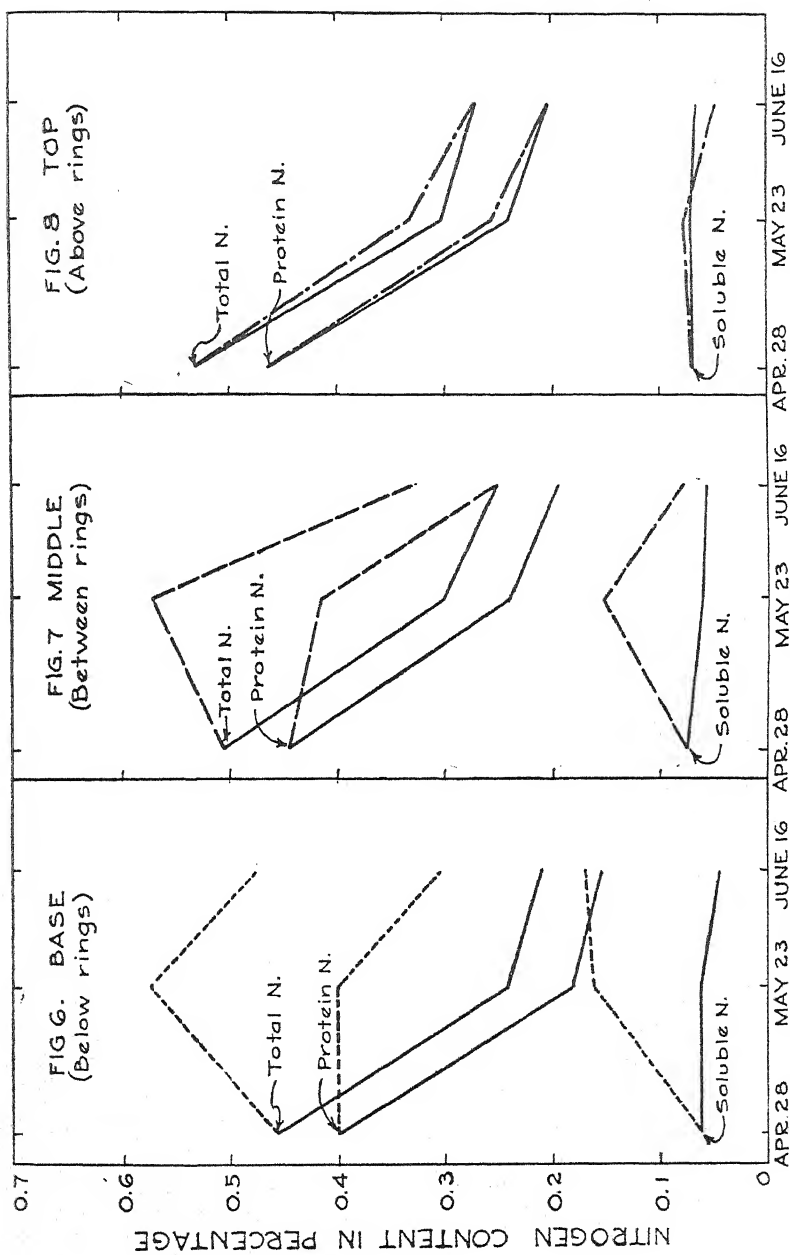
Composition of Leaves from Ringed and Unringed Poplars.

Chemical fraction.	Mg. in 100 gm. fresh leaves.	
	Check (unringed).	Ringed on trunk.
Reducing sugars	1242.7	1910.0
Sucrose	1875.3	1749.7
Total sugars	3118.0	3659.7
Starch	2142.5	4444.0
Total carbohydrates	5260.5	8103.7
Protein nitrogen	967.0	696.0
Soluble nitrogen	62.3	52.3
Total nitrogen	1029.3	748.3

The nitrogen analyses are difficult to explain on any basis other than that of the normal upward movement of nitrogen in the phloem of these plants. The piling up of soluble nitrogen below the rings in the base segment (Fig. 6) and the rapid loss of nitrogen from above the rings (Fig. 8) both suggest such action. The failure of nitrogen to accumulate above the rings, a result contrary to the observations of Maskell and Mason (16) on the annual cotton plant, and the low nitrogen content of leaves grown on ringed branches (Fig. 2 and Table III), are considered to be further evidence of the upward translocation of nitrogen in the phloem in woody perennials. Thomas (22) reports that absorbed inorganic nitrogen is combined into non-protein organic forms in the roots of apple trees, and he implies that these forms are translocated upwards. The absence of inorganic nitrogen in the upper portions of our plants agrees with the observations of Thomas, and our nitrogen analyses suggest that the normal upward movement of nitrogen in these plants is in the organic form and in the phloem. On the other hand, the loss of nitrogen from between the rings in the latter part of the poplar experiment (illustrated in Fig. 7)



Figs. 3-5. Fig. 3. Carbohydrates of the bark of the base segment. Solid line represents check and broken line, ringed trees. Note normal loss of carbohydrates from ringed treatment. 4. Carbohydrates of the bark of the middle segment. Note retarded loss of total carbohydrates from between the rings (broken line). 5. Carbohydrates of the bark of the upper segment. Note the rapid accumulation of carbohydrates above the rings after leaves were formed (broken line).



Figs. 6-8. Fig. 6. Nitrogen of bark of base segment. Note accumulation of soluble nitrogen in ringed treatment and loss of protein nitrogen in last period. 7. Nitrogen of bark of middle segment. Note early accumulation of soluble at the expense of protein nitrogen and the rapid loss of total nitrogen from between the rings in the last period. 8. Nitrogen of bark of upper segment. Note normal loss of nitrogen to buds and leaves and failure of protein nitrogen to accumulate above ring.

cannot, like the carbohydrate loss shown in Fig. 4, be explained on the basis of respiration, and suggests that the retention of organic materials by the living cells of the phloem is relative only, and that under some conditions organic nitrogen, and presumably other organic compounds, may escape into and move in the xylem, where their presence has been reported by numerous observers.

The accumulation of organic nitrogen below the rings at the same time that carbohydrates were being lost, presumably to the roots, is of interest, as it bears upon the problem of the mechanism of translocation. The Münch hypothesis supported by Crafts (2) does not permit of simultaneous translocation in the phloem of two materials in opposite directions, a feat which would appear to have been achieved in this material.

The synthesis of organic forms of nitrogen in the roots of plants should depend upon liberal carbohydrate supplies in these organs, and theoretically it should be possible to so starve the roots of an apple or other tree that not all of the inorganic nitrogen absorbed would be combined in the roots. In this condition the apple should behave like cotton (16). One of the large apple trees used for the data in Fig. 2 and and 10A was so closely ringed and cut for chemical samples in 1932 that only one small, partially shaded limb was left to supply carbohydrates to the roots of the tree in 1933. Ringed limbs on this tree showed an abnormally long new growth in comparison with the ringed limbs of the other trees in the experiment, and contained nearly as much nitrogen as the normal, unringed limbs. The growth and nitrogen data for this starved-root tree are compared with check and ringed branches on normal trees in Table IV. It is possible either that organic nitrogen had leaked from the weakened root parenchyma into the xylem, as indicated in Fig. 7, or that the low carbohydrate level of the roots had prevented the synthesis of organic nitrogen in the roots.

TABLE IV.

Sprout Growth and Composition of the Bark of Apple the Second Season after Ringing.

Measurement.	Check limbs (Av. of 4).	Ringed limbs on normal roots (Av. of 4).	Ringed limb on starved roots (one only).
Apical growth (mm.)	37.0	2.5	21.5
Sucrose (%)	3.62	4.26	8.0
Soluble nitrogen (mg. in 100 gm.)	17.2	18.7	20.0
Protein nitrogen (mg. in 100 gm.)	293.7	190.0	274.4

THE BALANCE OF APICAL AND DIAMETER GROWTH.

The Effect of Ringing upon Diameter Growth.

The dependence of diameter growth upon food or other materials from active leaves has been shown by a number of workers (10), (12), (13), (21),

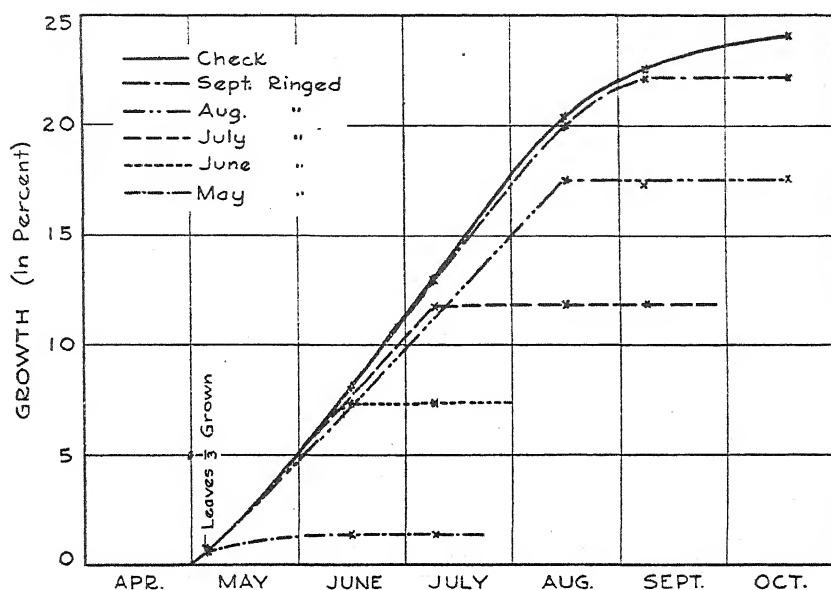


FIG. 9. The effect of ringing upon the diameter growth of apple. 1929 experiments. Diameter growth of the trunk was stopped abruptly by ringing to intercept translocation from the leaves.

and (24). The growth data from the 1929 experiments, plotted in Fig. 9, show very clearly that removing the phloem above a trunk segment stopped all diameter growth below the ring, irrespective of the time at which the treatment was given. The reduction in the diameter growth below the ring was immediate. Only in rings made in April or early May have we observed any measurable continuation of growth in these segments, and then only in regions isolated by ringing both above and below. Without the lower ring the foods responsible for the slight growth obtained between rings were apparently translocated downward. Growth has continued normally or at an accelerated rate above the rings, and an explanation of the data would seem to involve the sudden halting by the ringing treatment of the movement from the leaves of (a) a growth promoting substance of small bulk of the type frequently designated as a 'hormone', or (b) some essential cell-building constituent of greater bulk. The latter hypothesis is favoured by the author and will be discussed in more detail at a later time.

Growth data from the 1932 experiments are shown in Figs. 10 A and B. The trees used were ten to fifteen years old, and were handled like the smaller apple and the poplar trees, except that trunk segments were 12-16 in. long instead of 8-10 in. The base segments of the apple limbs differed also in being attached to the main trunk which was liberally supplied during the season of 1932 with food from unringed branches. In spite of this connexion the growth and chemical composition of these segments corresponded exactly to that of basal segments on the main trunk (Table V). The results of the growth measurement experiments may be summarized as follows:

(a) Diameter growth of the trunk started only after the leaves were well advanced in development.

(b) Segments above the rings on large trunks or branches made a normal or accelerated growth the first season after ringing in comparison with corresponding segments of untreated trees. The top segments of small trees made a more limited growth, probably because of nitrogen deficiencies.

(c) Segments below the lowest ring made no diameter growth after the ring was cut. As will be shown later, these segments sprouted freely.

(d) Segments isolated by two rings made little or no diameter growth, but tended to sprout freely in the spring.

(e) Segments isolated by double-ringing but with foliage permitted to develop on branches attached to the segment, centre-branch treatment, made a diameter growth which was proportional to the leaf area developed. Defoliation of these attached branches prevented diameter growth but did not prevent apical growth or sprouting in the isolated segment.

The Effect of Ringing upon Apical Growth.

In contrast to the practically complete inhibition of diameter growth on ringed segments, the growth of apical buds on attached branches has shown little, if any, check, and the stimulation of dormant and adventitious buds has been striking. All thrifty trunk segments, except those already carrying a large, active leaf surface, have shown this tendency toward the dominance of apical over secondary growth. The negative correlation between total diameter growth in percentage and the number of dormant and adventitious buds growing on each trunk segment is shown in Fig. 11. The segments classed as having had few or no sprouts were connected by unbroken phloem to active leaves. It should be pointed out that these leaves were produced by growing buds, so that the sections indicated as non-sprouting had grown normally from their upper buds, and, as a result of leaf production, subsequently began diameter growth without further

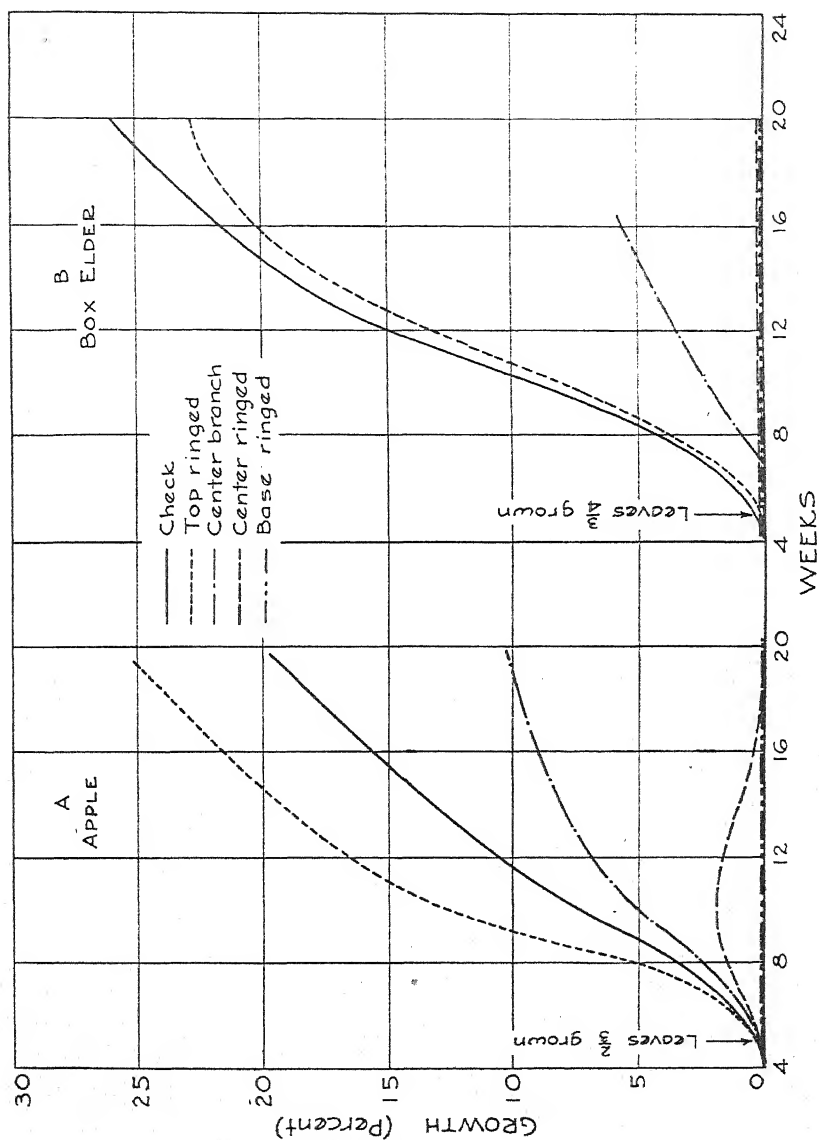


FIG. 10. The effect of ring-banding upon the diameter growth of large apple and box elder. 1932 experiments. Diameter growth was dependent upon developed leaves.

sprouting, while the sections isolated from active leaf area by ringing and removal of growing buds, continued sprout production. The hypothesis that stored food is used preferentially in the production of sprouts and leaves is clearly supported.

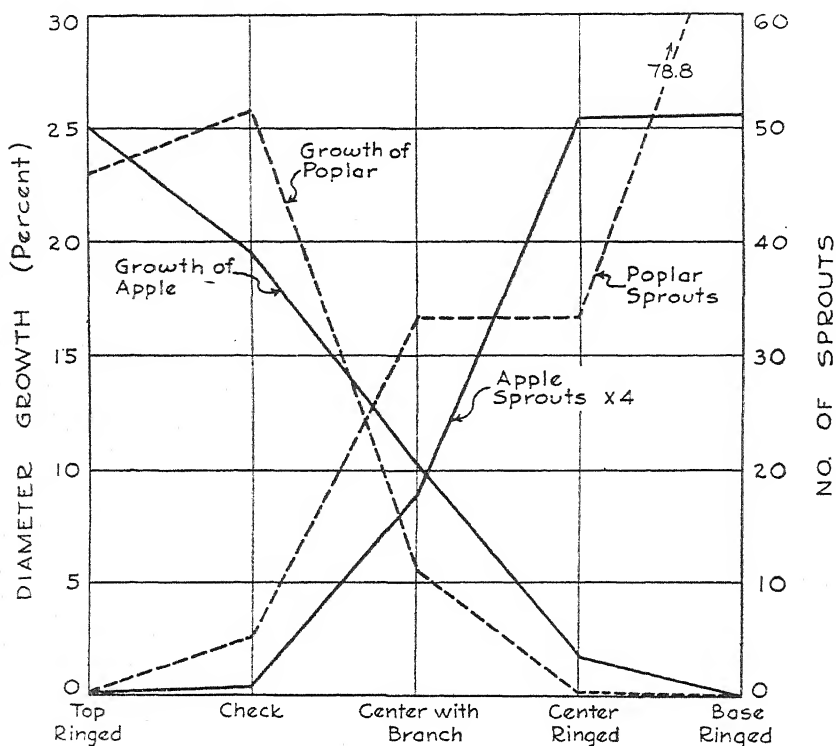


FIG. 11. The negative correlation between sprout and diameter growth. The treatments which gave the greatest diameter growth resulted in the fewest sprouts per segment.

Analyses of the various trunk segments (Fig. 12), showed that the composition of the check and upper segments at the time the buds were starting, corresponded to the composition of the sections between and below the rings at the later time when they were producing adventitious sprouts. What we may call the 'sprouting condition' was prolonged until June 16 by ringing two-year-old poplars, and presumably could have been maintained until the food reserves of the sections were exhausted. All sprouts were counted and removed at weekly or shorter intervals, so that the large numbers reported do not represent a large or exhaustive growth, and cannot be used to explain the loss of nitrogen from between the rings shown in Fig. 4. In this particular material the middle segments showed some sprout and callus growth in early May, but had stopped sprouting in June when the nitrogen was being lost.

Growth 'Hormones'.

The concept of hormone control of plant development has been borrowed from animal physiology, and ordinarily involves the idea of control by minute quantities of active substances. According to Went (23) and Bakheuyzen (1) these growth-controlling substances of plants are produced in the apical meristems. Branched trunk segments on 20 two-year-old poplar trees were used to check the hormone hypothesis. These were isolated by ringing and allowed to develop normally except that all leaves were removed when one-eighth grown or smaller. Over a period of six weeks a large number of buds made an essentially normal apical growth, decreasing with the exhaustion of the food, and particularly the nitrogen supply (Table I), but none of the trunk segments made any measurable diameter growth. Similarly, ringed segments with the leaves left on made a normal diameter growth in the first weeks of the growing season. Proebsting (21) has checked this same point with microscopic observations of cambial activity on defoliated branches. The data seem to require the conclusion that 'hormones' produced in apical meristems are not capable of inducing cambial growth in woody stems, even though, as at the beginning of our experiments, apparently adequate supplies of carbohydrate and nitrogenous foods are available from storage. The possibility of 'hormones' produced in active leaves is of course not excluded.

Chemical Composition and Growth Balance.

More than one hundred wood and bark samples have been analysed as outlined under materials and methods. Duplicate samples of bark and wood were collected (*a*) at the time bud growth was starting, (*b*) at the time, some two or three weeks later, that cambial growth was starting, and in the case of poplar, a third collection was made on June 16, six weeks after the buds opened. The collections were planned to show particularly the composition of the various trunk segments at the time that tendencies towards apical or diameter growth had been fixed by the treatments. The data for the poplar wood and bark have been presented in Tables I and II and Figs. 3 to 8. The sugar and nitrogen analyses of the wood and bark of box elder and large (fifteen-year-old) apple trees with varying treatments are given in Table V.

Sprouting is shown in Fig. 11 to have been dominant in the segments below or between the rings, and cambial growth dominant above the rings, provided the attached leaves were sufficiently developed. Soluble, organic nitrogen compounds have been consistently correlated with the tendency toward sprouting. The data of the 1932 experiments (Table V) emphasize the uniformity with which cambial activity was correlated with relatively

low soluble nitrogen and sprout growth with a relatively high percentage of this fraction. Absolute values varied with the species, but the changes with growth type were consistent in all cases studied. The lack of any consistent correlation between insoluble (protein) nitrogen or sugars and growth type may also be observed in the Table. The utilization of protein reserves in new growth is indicated, but is less striking than was observed in the smaller poplar trees (Tables I and II).

TABLE V.

A Comparison of the Chemical Composition of Tree Sections showing Sprout and Cambial Growth. (All data as mg. in 100 gm. fresh weight.)

Species and section.	Sucrose.	Reducing sugars.	Protein nitrogen.	Soluble nitrogen.	Type of growth.
<i>Wood Samples.</i>					
Apr. 8, ck., elder	415	65	85.8	26.8	Sprout
May 14, ck., elder	265	70	73.6	14.7	Cambial
Between rings, elder	230	10	76.4	37.7	Sprout
Below rings, elder	225	15	74.4	40.4	Sprout
Above rings, elder	173	77	64.0	14.1	Cambial
Between rings, apple	156	110	65.0	26.7	Sprout
Below rings, apple	148	142	84.5	27.0	Sprout
Above rings, apple	101	245	95.0	4.9	Cambial
Above rings, apple	73	193	41.6	4.6	Cambial
<i>Bark Samples.</i>					
Apr. 8, ck., elder	1220	170	454.6	223.6	Sprout
May 14, ck., elder	1060	120	400.6	118.3	Cambial
Between rings, elder	860	10	583.2	244.4	Sprout
Below rings, elder	673	7	445.2	236.5	Sprout
Above rings, elder	1460	155	436.4	147.0	Cambial
Between rings, apple	520	605	259.6	54.2	Sprout
Below rings, apple	400	605	199.2	41.1	Sprout
Above rings, apple	245	895	139.5	11.9	Cambial
Above rings, apple	270	775	177.5	11.4	Cambial

All of the data on soluble nitrogen are brought together in Fig. 12, and they show that in every case the sprouting trunk segments were relatively high in this material, while the segments showing cambial growth contained relatively low percentages of this fraction. The bark with its conducting tissues and lower percentage of inert materials shows more strikingly a condition which was present in the wood also. In both wood and bark in every comparison studied :

(a) Soluble nitrogen tended to be high in the spring at the time when the buds were starting.

(b) It dropped with the initiation of leaf activity and was relatively low in all tree segments showing cambial growth.

(c) Interference with leaf connexions by defoliation or by ringing resulted in a sharp rise in soluble nitrogen and in the initiation or resumption of sprouting.

Fractionation of the soluble nitrogen into inorganic, amide, amino, basic, and 'other' nitrogen showed no organic fraction consistently correlated with sprouting in all species. The data indicated that the various

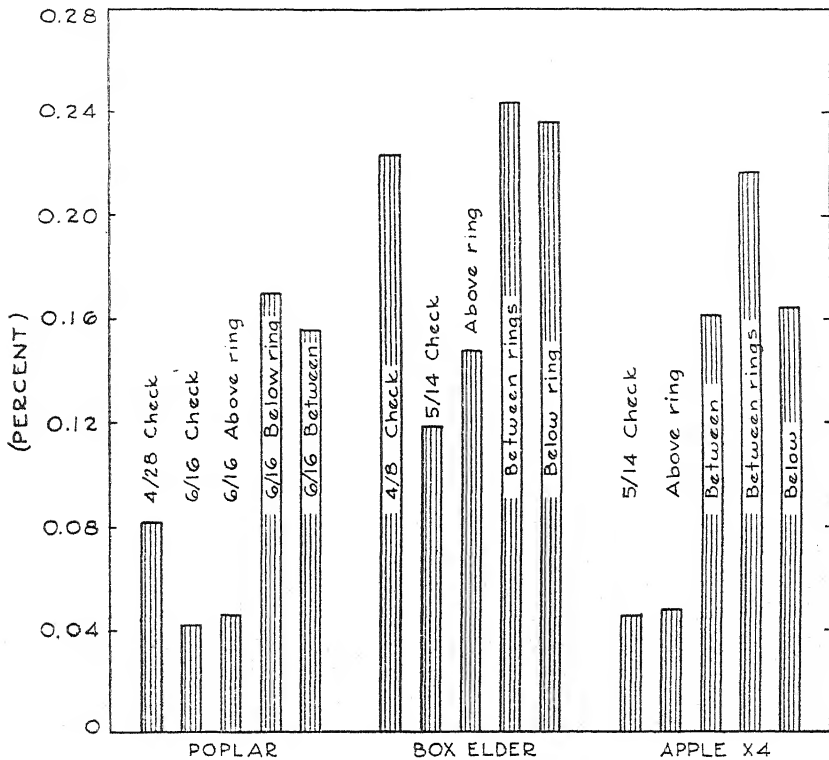


FIG. 12. The relation of soluble nitrogen to sprout and cambial growth. The early checks and the segments between and below the rings showed sprouting and high soluble nitrogen. The late checks and the segments above the rings showed cambial growth and low soluble nitrogen.

soluble, organic materials were more or less interchangeable in their effect of stimulating sprouting. We have, then, the possibility that non-protein, organic nitrogen materials, whether synthesized in the roots or formed by the digestion of stored proteins, are directly utilizable only by apical or similar meristems. Such a conclusion would not be without logical support, for the massed, apical meristems should be best able to maintain conditions favourable for the condensation of the simple nitrogen compounds into proteins and protoplasm. Bakheuyzen (1) reports that apical meristems have a pH nearer the average isoelectric points of the protoplasm proteins than is possessed by the other tissues of the plant, a condition which should favour condensation reactions. Evidence that the shape of the meristem is a factor in the utilization of soluble, organic nitrogen compounds is given

by the development of callus growths at the rings of 'middle' and 'base' segments. These segments made no measurable cambial growth, but produced both sprouts and callus from stored foods.

Maskell and Mason (17) found a large proportion of the organic nitrogen of cotton moving from the leaves in the form of proteins. We can build the second part of our hypothesis on this observation, and assume that the cambium has a lower condensing power than the massed meristems, and consequently is able to form new cells only when supplied with protein materials freshly condensed in active leaves. After these fresh proteins have once been stored they would seem to be re-utilizable only in the simpler, digested forms which favour apical or sprout growth. In the case of cambial growth on stored nitrogenous foods in the 'top' segment of ringed trees, we may assume that the digested food was moved to the leaves, condensed, and returned to the growing regions.

This outline is obviously hypothetical, but it fits the observed facts. Defoliated trees cease cambial development almost at once. If they contain reserve proteins, these are digested, the soluble organic nitrogen content of the tissue rises, sprout growth is initiated, and the lost leaf area is replaced; after which cambial activity may be renewed. This explanation appears better than hypotheses based upon hormone action because of the quantitative nature of the responses. With half of the normal leaf area remaining on a given tree segment we may expect something like half of the normal cambial growth. (Fig. 10, growth of 'side branch' segments.) The direct proof that cambial growth depends upon mobile supplies of condensed proteins is difficult to obtain because of the impossibility of distinguishing between stored and moving proteins in the trunk sections with the present methods of analysis, and because stored proteins are still being used at the time when our hypothesis assumes that fresh proteins are being moved into the tissue and used in cambial growth. Our analyses have shown, however, that insoluble (protein) nitrogen disappears from the trunk in the spring under sprouting conditions, and it may be assumed to have accumulated late in the previous season under conditions favouring cambial growth. The loss of protein from the trunk segments was accompanied by an accumulation of non-protein nitrogen and sprouting. A reduction in non-protein nitrogen, such as would result from condensation in the leaves, has been shown by segments with actively developing cambium. As suggested above, the first supplies of condensed materials may be used by the cambium while moisture and other factors are favourable for growth (14), and new storage be deferred until midsummer or fall. Carbohydrate materials from the leaves are ignored in forming this hypothesis because of the very high sugar content of all samples during the early sprouting period when no cambial activity was observable, and the normally lower content during the period of cambial development.

Translocation and Polarity.

When a limb segment of apple carrying a foliage-bearing branch in the middle of the segment was isolated by ringing, so that all food materials were confined to the segment, cambial growth on the main axis was 200 per cent. greater below the food-supplying branch than above it. Also the bases of ringed limbs attached with uninterrupted phloem connexions to rapidly growing trunks did not in any case show cambial growth, although they sprouted freely (Figs. 10 A and 11). This tendency for secondary growth to extend downward along the stem is recognized in pruning practice, where the larger wound made by close cutting is found to heal faster than a small wound an inch or two further from the main axis. Chemical analyses showed that segments below rings gained in total, and particularly in soluble, nitrogen, while they lost in carbohydrates (Figs. 3 and 6), while segments above the rings lost nitrogen and gained in carbohydrates (Figs. 5 and 8). The tendency of the secondary growth materials to move to the lower end of a stem segment might favour root growth as well as cambial growth and account for the polarity in the development of these organs generally exhibited by plants.

DISCUSSION AND SUMMARY.

1. Phloem rings on woody plants were found to check temporarily the movement of both carbohydrate and organic nitrogen compounds. With periods of four to six weeks the effectiveness of the rings seemed to disappear. The suggestion is made that the cells of the ringed segments became increasingly permeable so that organic materials were lost into the xylem and thus moved past the interruption in the phloem.

2. In all of the plants studied, from two-year-old poplar trees to fifteen-year-old apple trees, only organic forms of nitrogen were found in the tops, and the movement of nitrogen was checked by ringing. In a single test the movement of total ash did not appear to be affected by ringing. These data, together with the observations of other workers, would seem to indicate that the organic or inorganic form determines whether or not nitrogen, and presumably other elements such as phosphorus, move normally in the phloem or in both the phloem and xylem. Curtis (6) worked with woody plants similar to those used here. Most of his plants were grown in full sunlight and on moderately rich soil, so that that they should have contained a considerable accumulation of carbohydrates in the roots. Under these conditions, and with these plants, nitrogen is apparently synthesized to organic forms within the roots (22), and moved upward normally in the phloem. Maskell and Mason (16) worked with cotton, which is grown as an annual, and they found the synthesis of nitrogen to

occur in the leaves from inorganic nitrogen moved upward in the transpiration stream. The failure of the cotton plant to synthesize absorbed nitrogen in the roots may have been due to low carbohydrate supplies in these organs or to physiological factors. The ability of the inorganic nitrogen to move in the transpiration stream while the organic forms are normally transported in the phloem may be explained upon the basis of the normally greater permeability of the cell membranes of green plants to ions, so that nitrogen ions may escape from the pericycle into the non-living xylem elements when the un-ionized, organic compounds must confine their intercellular movements to the plasmodesma and the perforations of the sieve plates. The permeability of cell membranes is, however, capable of considerable variation, and cells normally impermeable to a given compound may become permeable as a result of freezing or other non-lethal injury. The disputed question of whether nitrogen moves upward in the xylem or phloem would thus depend first, upon the form of the nitrogen, and second upon the condition of the living cells bordering the xylem.

The author interprets his data as indicating that nitrogen synthesized to organic forms in the roots is normally moved upward in the phloem, but that the escape of organic nitrogen into the transpiration stream is not impossible, and is to be considered the cause of the observed loss of total nitrogen from isolated trunk segments in the experiments reported here.

3. Secondary growth in the limbs and trunks of woody plants is shown to be dependent upon active leaf area with uninterrupted phloem connexion to the growing segment. Secondary growth was not initiated until the first new leaves were one-third grown or larger, and it was stopped at once by defoliation or by ringing to interrupt downward movement from the leaves. The estimate of one-third grown leaves at the time that cambial growth started is based upon normal leaf size, and the first leaves formed from a bud are frequently not more than one-third of normal size at full maturity. There would seem to be no question that photosynthetic activity in the leaves precedes normal cambial growth. On the other hand, carbohydrate levels were higher in the sprouting segments than in those with active leaves which showed cambial growth, so that the formation of available carbohydrates by the leaves cannot be used as an explanation of their relationship to cambial development.

4. Primary growth was preceded by an accumulation of the simpler forms of organic nitrogen, and was apparently dependent upon them. The suggestion is made that simple organic-nitrogen compounds can be condensed to protoplasmic proteins in the massed and compact apical or callus meristems, or in the leaves, but not in the thin layer of the cambium. If, as is generally assumed, the partially denatured stored proteins can be moved only after digestion to simpler forms, they would appear then to be used directly only by multicellular or massed meristems as contrasted

with the one-celled or few-celled cambial layer. This hypothesis requires the assumption, which is supported by the data of Maskell and Mason (17), that freshly condensed proteins are moved from the leaves without digestion.

The following outline of nitrogen movement in woody plants is suggested: (a) inorganic nitrogen is absorbed and synthesized into soluble organic forms by the roots, provided these organs are adequately supplied with carbohydrates; (b) permeability relationships normally confine the upward movement of these materials to the phloem; (c) soluble, organic nitrogen compounds may be used directly by the apical meristems of the top and presumably of the roots, or, (d) they may be moved to the leaves, condensed into more complex forms and returned via the phloem to be used in either secondary growth or storage; (e) stored proteins are made available by digestion to soluble forms which then behave like soluble forms from the roots.

5. The growth materials moving from the leaves have shown a definite tendency to move and act downward. The materials accompanying primary or sprout growth show a tendency to move and act upward. There seems to be some probability of these movements being connected with polarity. The primary rooting of cuttings involves massed apical root meristems, and is, of course, possible without attached leaf area, provided stored food materials are present, but unpublished data from this laboratory indicate that active leaves have a direct and important effect upon certain types of root growth which is analogous to the effects reported here, and entirely apart from the question of total food available. In many plants the polarity of bud development is more strongly fixed than that of rooting, a condition which might be due to a directional movement of soluble organic nitrogen compounds.

6. The absence of any effect from developing apical meristems freed of leaves, and the quantitative nature of the relation of leaves to secondary growth would seem to exclude the action of 'hormones' as formerly conceived. If 'hormones', produced in the leaves and acting quantitatively rather than qualitatively, are considered to be concerned, it is felt that the designation of these hormones as 'possibly condensed proteins' has certain merits as a working hypothesis.

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Energy Absorption by Leaves in Normal and Plane Polarized Light.

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With one Figure in the Text.

INTRODUCTION.

IN investigations of the effect of plane polarized light on chemical processes and biological phenomena it is usually uncertain whether the energy absorbed from the two types of radiation is the same. In view of the work of Dastur and Asana (4) on the formation of carbohydrates in leaves it might be surmised that the energy absorbed from polarized and non-polarized light is not different, since no accelerating influence of the polarized light was noticed. It would seem, however, of interest to determine directly the energy absorbed by a leaf from polarized and non-polarized light of equal intensity.

The energy absorbed by the leaf is determined by the absorption of a thermopile with and without the interposition of the leaf. The main sources of error are the reflection of light by the leaf surface and the scattering of light after it has passed through the leaf so that all the transmitted light does not reach the recording instrument. Warburg and Negelein (8) have overcome the difficulty of measuring the amount of light absorbed by using thick suspension of an alga in a flask with silvered sides so that the whole of the incident light is absorbed, but this device is not practicable in the case of a leaf.

It has been pointed out by Reinke (5) and Briggs (1) that some of the light energy is absorbed by the colourless portions of the assimilating organ, but here we are concerned with the coefficient of absorption of the leaf as a whole.

The coefficient of absorption of different leaves in sunlight was

measured by Brown and Escombe (2) and they found that it varied in leaves of different plants from 0.686 to 0.774. They also noticed that a green leaf was very selective in its absorbing power, as the light did not decrease in geometrical proportion as leaves were superimposed in arithmetic proportion. Similar results were obtained by Timiriazeff (7) with chlorophyll solutions extracted from a unit area of one maple leaf. Brown and Escombe (2) also found a very small increase in the value of the coefficient of absorption with increase in age of leaves. They also noticed that the coefficient of absorption of the white portion of the leaf was less than that of its green portion.

INVESTIGATIONS.

Arrangement of the Apparatus.

Transmitted plane polarized light and a beam of normal light of equal intensity side by side are obtained as described below. A wooden box of the shape of a truncated pyramid, with the smaller side measuring 18 in. square and the larger side 24 in., is used (Fig. 1 A). The box is divided equally into two compartments by a vertical partition traversing the length of the box. The two compartments are used alternately as chambers for plane polarized and normal beams of light. At the broader face of the pyramidal box there is a hinged door to cut off all extraneous light. To obtain plane polarized light a pile of one to eight glass plates is kept at the Brewsterian angle in one of the chambers, while a similar number of plates is kept perpendicularly to the incident beam in the other chamber. The positions of the plates are then reversed in the two chambers to verify the results obtained in the first determinations.

At first, attempts were made to measure the absorption by leaves of light from a 1,500 watt Phillips gas-filled lamp, but it was found inadequate. It was necessary to use a source of very high light energy; this was achieved by the use of a flood-light lamp, of Novalux Projector type (General Electric Co., U.S.A.), with a 1,000 watt bulb. This lamp gave a very intense beam of light by virtue of its parabolic reflector. Another important requirement was incidently fulfilled by this lamp in giving a parallel beam of light. In order to obtain the maximum polarization the rays must strike the glass plates normally and a parallel beam of light is essential. The flood-light lamp is kept at the smaller face of the wooden box so that both the compartments are equally illuminated.

A vertical water-cooler with glass surfaces and of 2 in. thickness is used, in which the circulation of water from a water-tap is maintained at a fairly rapid rate (Fig. 1 A) by means of the inlet and outlet tubes.

A microthermopile in conjunction with a D'Arsonval galvanometer was used for measurement of energy. All the measurements of light intensities are recorded as galvanometric deflexions.

The exact position at which the microthermopile should be kept in the two chambers so as to have equally intense beams are first fixed. A piece of thin cardboard was cut to the shape and size of the front portion of the microthermopile with a hole in the centre exactly corresponding to

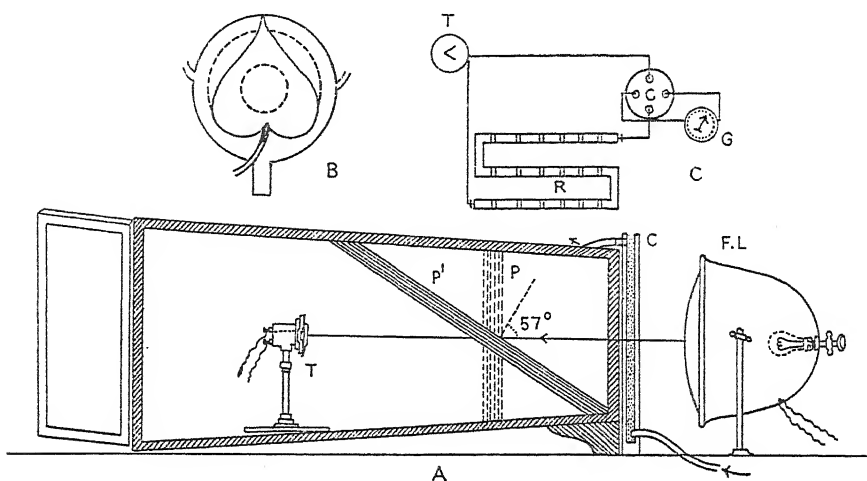


FIG. 1. A. A diagrammatic sketch of the apparatus for obtaining normal and plane polarized beams of light of equal intensities. B. Sketch of the face view of the leaf attached to the microthermopile. C. Sketch showing the connexions.

G = galvanometer. R = resistance. C = water-cooler. F.L. = flood-light lamp. P = vertical glass plates. P' = inclined glass plates. T = microthermopile.

the hole in the microthermopile. The disc can be fastened on to the microthermopile by means of rubber bands. The leaf is pinned to the disc by means of drawing pins (Fig. 1 B). The disc can be slid back when the total intensity of the incident beam is to be measured. After this is noted by the deflexion on the scale, the disc is slid into position on the face of the thermopile. The use of elastic bands ensured the closest approach of the leaf to the face of the thermopile so that the intensity of light first recorded by the thermopile is also that of the light incident on the leaf. The close approach further ensures the non-interference of light scattered from other portions of the leaf-surface receiving light. The hole in the disc also corresponds to that in the thermopile face, thus cutting off all extraneous scattered light. The reading on the scale of the deflexion of the galvanometer is again taken. The difference between the first and second reading gives the amount of the light energy absorbed by the leaf and the total incident light energy (first reading) divided by the amount of light energy absorbed by the leaf is the coefficient of absorption of that leaf.

The thermopile with the disc and leaf attached is placed in the second chamber. The total light intensity incident is made equal to that used in the first chamber and the process is repeated. Thus the amounts of light

energy absorbed by the same region of the leaf from the polarized and normal beams of light of equal intensities are determined and the coefficients of absorption in the two cases calculated.

As the intensity of light is very high, the current generated by the microthermopile is reduced by means of a resistance of 410 ohms in series. When the light transmitted by the leaf is measured the resistance is short-circuited, thus giving a full deflexion of the thermopile.

The sensitivity of the galvanometer, i.e. the current required to produce 1 mm. deflexion is determined according to the method of Starling (6). It is 0.03458 micro-amperes.

Measurement of the Percentage of Polarization Obtained by a Pile of Glass Plates.

The following Table I gives the percentage of polarization with one, three, five, and eight glass plates, determined by the method of Wood (9).

TABLE I.

No. of glass plates	1	3	5	8
Percentage	21	55	71	89

It will be seen that the percentage of polarization is fairly high with eight plates.

Method of Avoiding Temperature Effects.

It is absolutely necessary to avoid the heat rays in order to obtain accurate results of the light intensity. At no stage of the experiment was the light kept on for a longer period than a minute and a half, which period was necessary for taking the reading on the scale. The temperature measurements of the two chambers were made by means of a Beckmann's thermometer in order to determine if the heat rays were completely absorbed by the water-cooler. It is also necessary to study the temperature differences in the two chambers. The light intensity measurements before and after *thirty minutes* lighting period with the microthermopile were taken in the two chambers, and it was found that the rise in temperature in the two chambers was only 0.02 C. Thus heat-interference with the intensity measurements was completely eliminated. A well-regulated fan was kept working for the rapid circulation of air in the room.

The whole apparatus is shown in Fig. 1, but not the vertical partition. The light after passing through the water-cooler enters the water-chamber, and the light in both the chambers passes through the same number of plates (1, 3, 5, or 8 as the case may be) but with a difference in their position.

The leaves of twelve different species belonging to twelve different

families were taken for studying the absorption of light energy from polarized and normal beams of light. As the incident light intensity is the same in all cases the values of the light transmitted are given below (Table II) in terms of deflexions of the galvanometer. The light transmitted by the leaf in the normal beam is greater than in the polarized beam, the difference in the two beams being always positive. Only the fourth set of determinations made with a pile of eight plates, giving 89 per cent. of polarization, is given in Table II.

TABLE II.

The Intensity of Light Transmitted by Leaves with Normal and Polarized Light Beams.

The intensity is given in terms of galvanometer deflexion (mm.) Full light = 62 mm. Eight plates used, giving 89 per cent. polarization.

	Left chamber with polarized beam.			Right chamber with polarized beam.		
	Normal.	Polar- ized.	Differ- ence.	Normal.	Polar- ized.	Differ- ence.
<i>Bougainvillea spectabilis</i>	42.0	33.5	8.5	41.0	29.5	11.5
<i>Stachytarpheta indica</i> . .	46.5	36.5	10.0	44.5	29.5	8.0
<i>Crossandra undulaefolia</i> .	44.5	34.5	10.0	42.0	32.0	10.0
<i>Poinsettia pulcherrima</i> .	45.5	35.0	10.5	45.0	34.5	10.5
<i>Musa sapientum</i> . . .	40.5	31.5	9.0	38.5	28.5	10.0
<i>Bauhinia</i> sp.	46.5	37.0	9.5	45.0	36.0	9.0
<i>Vitis quadrangularis</i> .	42.5	31.0	11.5	38.5	28.5	10.0
<i>Abutilon asiaticum</i> . .	50.5	43.5	7.0	43.5	32.5	11.0
<i>Polyalthia longifolia</i> .	47.5	38.5	9.0	52.0	42.0	10.0
<i>Bignonia purpurea</i> . .	44.0	34.5	9.5	45.0	36.0	9.0
<i>Mangifera indica</i> . . .	51.5	40.0	11.5	50.0	39.5	10.5
<i>Ficus elastica</i>	39.5	28.5	11.0	37.0	28.5	8.5

In order to study the differences between the absorption of light from the two beams with the four different percentages of polarization, the mean of the two sets of readings (right and left chambers polarized alternately) is taken and the differences (N-P) with the four different percentages of polarization are given in Table III. The results show that with the polarization obtained with one glass plate, the difference (N-P) is very little, while with three, five, and eight plates the differences have greatly increased, they are more or less equal with the higher percentages of polarization. The influence of the polarized beam in the absorption of light energy by the leaves becomes marked with higher percentages of polarization.

The study of the results reveals a point of interest. It is seen that the amount of light transmitted by a leaf decreases as the number of plates is increased. This holds good both for normal beams and polarized beams in the four sets of determinations (Table IV).

TABLE III.

The Absorption Differences (N-P) in mms. with Varying Percentages of Polarization.

Total intensity of light = 62 mm. (with 410 ohms as extra resistance).

Mean values.

	One plate (21 %)	Three plates (55 %)	Five plates (71 %)	Eight plates (89 %)
<i>Bougainvillaea spectabilis</i>	3.0	9.7	8.2	10.0
<i>Stachytarpheta indica</i>	4.2	14.2	9.0	9.0
<i>Crossandra undulataefolia</i>	3.7	12.5	10.5	10.0
<i>Poinsettia pulcherrima</i>	1.0	12.2	9.5	10.2
<i>Musa sapientum</i>	4.0	11.5	10.2	9.7
<i>Bauhinia</i> sp.	5.0	15.2	9.5	9.2
<i>Vitis quadrangularis</i>	1.0	10.2	8.2	11.2
<i>Abutilon asiaticum</i>	4.9	12.7	9.7	9.0
<i>Polyalthia longifolia</i>	4.0	12.5	7.0	9.5
<i>Bignonia purpurea</i>	6.0	11.0	8.7	9.2
<i>Mangifera indica</i>	6.0	10.5	8.5	10.1
<i>Ficus elastica</i>	5.7	10.0	8.7	9.7

TABLE IV.

Light Transmitted by Leaves as the Number of Plates is Increased (mm. Deflexion).

Mean values.

No. of plates. Normal beam. Polarized beam.

Bougainvillaea spectabilis.

1	72	69
3	60	50
5	49	40
8	42	31

Stachytarpheta indica.

1	74	70
3	70	56
5	54	45
8	45	37

This fact appears very curious, as the total intensity of the light falling on the leaf was the same in all cases, whatever the number of plates. The decrease in the amount of light transmitted with the increasing number of plates might be attributed to the greater absorption of the heat rays, but in view of the measurements of the temperature in the polarized and normal chambers given above this does not appear likely. In order to verify the point a solution of ammoniacal copper sulphate was placed after the plates to absorb all the heat rays. The solution had the transmission range of 4050 to 4360 Å.U.; there was thus no chance of any heat rays passing through the filter. The measurements of the light transmitted by

a leaf were then made, using different numbers of plates, and the same decrease in the light transmitted occurred. It is likely that a change in the quality of light is brought about when the number of plates is increased from one to eight. The relative intensities of the different rays are thereby altered and the differences in the total absorption of a leaf may then be due to the differential absorption power of the leaf for different rays.

From the values of the light transmitted by the leaves it is possible to calculate the coefficients of absorption. Brown and Escombe (2) have made direct determinations of the coefficient of absorption by means of a Callendar platinum thermometer. Their method was open to objections for more reasons than one, while the method adopted here in determining the light intensity is delicate and accurate.

It was not possible to measure the light reflected from the leaf surface, but a deduction in the values of the energy absorbed by the leaves can be made according to the observations of Coblenz (3), who measured the light reflected from the leaves. He found that for normal green and mature leaves 20 per cent. of the incident light is reflected. The leaves of the first ten species are green and mature, while of the last two plants, *Mangifera indica* and *Ficus elastica*, are green but with polished surfaces. It is possible that light reflected from these leaves is greater than 20 per cent. So the coefficients of absorption for the last two plants are smaller than those given in the table. It is not possible to apply a special correction for the reflection from the polished leaves.

The total radiant energy absorbed is calculated by the difference between the incident energy and the energy transmitted. In calculating the coefficient of absorption (Table V), the value of incident light is taken after deducting 20 per cent. from the total incident energy.

These coefficients are calculated on a basis different from that of Brown and Escombe (2), who give the coefficient in terms of the total incident light without deduction for reflection.

TABLE V.

Coefficient of Absorption of Leaves in Normal Light and in Light 89 per cent. Polarized.

Mean values.

Name of plant.	Normal.	Polarized.	Name of plant.	Normal.	Polarized.
<i>Bougainvillaea spectabilis</i>	0.901	0.920	<i>Vitis quadrangularis</i>	0.903	0.929
<i>Stachytarpheta indica</i>	0.891	0.913	<i>Abutilon asiaticum</i>	0.888	0.909
<i>Crossandra undulaefolia</i>	0.897	0.920	<i>Polyalthia longifolia</i>	0.881	0.904
<i>Poinsettia pulcherrima</i>	0.893	0.917	<i>Bignonia purpurea</i>	0.899	0.916
<i>Musa sapientum</i>	0.906	0.928	<i>Mangifera indica</i>	0.879	0.905
<i>Bauhinia</i> sp.	0.891	0.912	<i>Ficus elastica</i>	0.909	0.932

The coefficients of absorption of the leaves of different species vary from 0.879 to 0.932. According to these results the leaves generally absorb

about 88 to 93 per cent. of incident light. The differences in the absorption of light by different leaves are significant and must be due to various factors such as thickness.

A difference in the absorption of energy by the leaves from the normal and polarized beams is quite clear, the coefficient of absorption for the leaves in polarized light being greater.

In all these calculations no allowance is made for the scattering of light transmitted by the leaves not reaching the recording instrument. But in this particular investigation the leaf surface is exactly superimposed on the face of the microthermopile, leaving practically no intervening space in between, so that there is very little possibility of light emerging from the leaf not being recorded.

CONCLUSIONS.

The results of this investigation show a definite increase in the absorption of light by the same region of a leaf from the polarized as compared with the absorption from the normal beam. Transmitted plane polarized light is not capable of being reflected; if it were, it would not be possible to obtain a higher percentage of plane polarized light with increase in the number of glass plates. So the light energy absorbed by a leaf from the polarized beam is greater still than the light energy absorbed from the normal beam, as in the latter about 20 per cent. to 50 per cent. of light is reflected by the leaf surface, according to its reflecting capacity.

At first it might seem that the reflection factor is out of question, as incidence in both cases is normal, but as the leaf surface is not regular it is easily seen that light will be scattered in all possible planes. This reflected portion is lost to the leaf in the case of the normal beam, whereas in the case of the polarized beam, this loss is absent, due to the physical incapacity of transmitted polarized light for reflection. A very small loss, however, does occur from the polarized beam as part of the non-polarized portion suffers reflection at the leaf surface and is lost. Such a loss would, however, only be 20 to 50 per cent. of the 11 per cent., which is the non-polarized portion of the polarized beam. In the case of the normal beam, taking into consideration the quality of the surface presented by the leaves, it might be safely assumed that about 75 per cent. of the reflected light is lost to the leaf.

It is very difficult to assign a reason for the greater absorption by a leaf of light from the polarized beam, except that there is selective absorption of light by a leaf from a polarized beam, and that it may be due to vibrations of light being in only one plane, which is not the case with a normal beam where the vibrations are in all planes and also are rapidly changing their planes.

The greater absorption of light energy seems in no way beneficial to

the activities of the leaf. This is evident from the results of Dastur and Asana (4), as there is no enhanced photosynthetic activity in the plant in the polarized light as compared to the photosynthetic activity in the normal light. Plane polarized light has neither a specific effect on the photochemical reactions occurring in the leaf, nor does it increase the speed of the chemical reactions involved by virtue of its greater capacity to be absorbed. Accordingly, the leaf in no way functions more vigorously in the polarized beam than in the normal beam.

SUMMARY.

An apparatus is devised to measure the coefficient of absorption in a leaf of light energy from plane polarized and non-polarized beams of equal intensities. Four different percentages of polarization are used.

The total light intensity and the light transmitted by a leaf in each beam is determined by means of a microthermopile in terms of the deflexion of the galvanometer.

Leaves of twelve different species are used. The results clearly show that the leaves absorb a larger amount of energy from the polarized light than from the normal light.

The coefficient of absorption for the leaves of each plant from the polarized and non-polarized light is determined; a deduction of 20 per cent. of the total incident energy is made for the loss of light energy due to reflection from the leaf surface. The coefficients vary from 0.88 to 0.93. The coefficient of absorption is higher with the polarized than the unpolarized.

Transmitted plane polarized light is incapable of being reflected from the leaf surface, and therefore the coefficient of absorption of a leaf in the polarized light is really higher than that given.

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The Structure of the Starch Layer in the Glossy Petal of *Ranunculus*.

II. The British Species Examined.

BY

JOHN PARKIN, M.A.

With nine Figures in the Text.

INTRODUCTION.

IN a former paper (4) it was shown that in the petal of *Ranunculus Ficaria* the starch layer, apparently two to three cells deep as viewed in transverse section (Fig. 1, *st.*), is in reality composed of only a single row of cells, the false appearance being due to the peculiar oblique growth of these cells as viewed in longitudinal section (Fig. 2, *st.*). Incidentally the petals of the three common buttercups of our meadows and pastures, viz., *R. acris*, *R. bulbosus*, and *R. repens*, were also examined for comparison. The starch cells have likewise in these species the oblique character of growth, but with this striking difference, that the direction of the slope is the reverse of that shown in the petal of *R. Ficaria*. This suggested that considerable variation may occur in the structure of the starch layer in the genus, and the British species have now been examined with this end in view.

All the British species of *Ranunculus* have yellow and glossy petals with the exception of those belonging to the *Batrachium* section—the white water-buttercups—which consequently are not dealt with in this paper. The glossy species as described in Bentham and Hookers' *Handbook of the British Flora* (seventh edition 1930) number thirteen. *Ranunculus reptans* L. has since been raised to specific rank (1, p. 21). All have been examined except this last, the petal of which is not likely to differ materially in structure from that of its close ally, the common and variable *R. Flammula*.

For the supplying of flowers of the rarer British species, such as *R. ophioglossifolius* and *R. flabellatus*, I am indebted to Mrs. Foggitt and Lady Davy. Again, through the kindness of Professor J. H. Priestley, the microtome work in connexion with this paper has been carried out by his

laboratory steward, Mr. A. Millard. To all the above I wish here to tender my grateful thanks, and especially to the last mentioned for the care and interest he has taken in the cutting of the sections.

TECHNIQUE.

Mature petals of *Ranunculus* are usually such flimsy structures as to render the cutting of their sections without distortion, especially in the longitudinal direction, a matter of considerable difficulty. Petals from flowers in the full-bud stage, that is just before expansion, can be dealt with more easily. Such material has consequently been largely used in this investigation. How far, it may be asked, will the structure of the slightly immature petal differ from that of the fully expanded one? From a detailed examination of the petal of *R. Ficaria* at all stages of development we think the difference to be but slight. When slope is present in the starch cells, its steepness may be a little augmented on full expansion.

The material was fixed in Flemming's chromo-acetic-acid solution, and the sections cut moderately thick, 10 to 12 μ . If cut thinner the starch granules are apt not to be retained *in situ*. The stains used were either Hanstein's aniline violet or light green and safranin. The former is quite good and simple to use, but has the disadvantage of soon fading. The latter, a double stain, though more difficult to manipulate, is fairly permanent, colouring the cell-walls green and the starch granules pinkish.

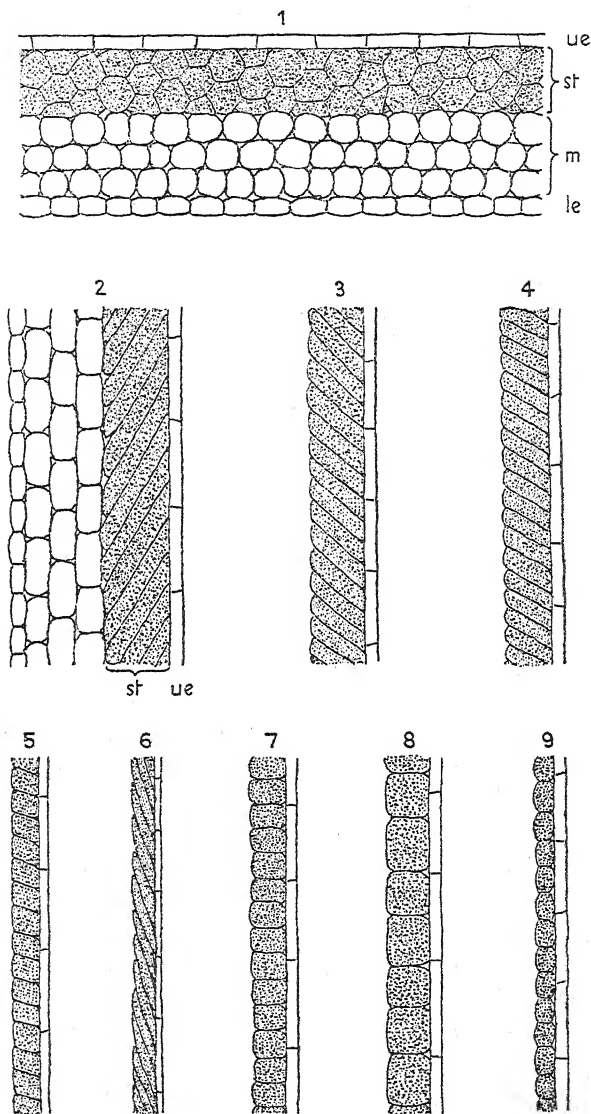
DESCRIPTIVE.

The species are taken in alphabetical order and the slope and direction of the starch-cells briefly described. In each instance the observations made refer to the middle part of the glossy (starch-containing) area of the petal. When slope is present it tends to be less pronounced at both the distal and proximal borders of this area. The degree of the slope is indicated roughly by the size of the acute angle the starch cell makes with the upper (ventral) surface of the petal. For example, an angle of 30 degrees indicates twice as much slope as one of 60 degrees. Regarding the direction of the slope the terms 'upwards' and 'downwards' refer respectively to the apex and base of the petal, and the terms 'outwards' and 'inwards' to the upper and lower surfaces. The measurements of the depth of the starch layer are approximate only and given for the sake of comparison.

R. acris L. The starch cells have a decided slope of about 45 degrees directed upwards and inwards. Depth of starch layer, 38 μ .

R. arvensis L. The slope is only slight, the angle being about 60 degrees. The direction is also upwards and inwards. The depth of the starch layer, 22 μ .

R. auricomus L. No slope is shown. The starch cells are almost



FIGS. 1-9. 1. Transverse and Fig. 2, longitudinal section of the petal of *Ranunculus Ficaria* L. 3-9. Longitudinal sections of various petals showing only upper epidermis and starch layer. 3. *R. bulbosus* L. 4. *R. repens* L. 5. *R. sardous* Cr. 6. *R. parviflorus* L. 7. *R. auricomus* L. 8. *R. Flammula* L. 9. *R. sceleratus* L. u.e., upper epidermis; l.e., lower epidermis; st., starch layer; m., mesophyll. Cell contents are only indicated for the starch layer. Magnification $\times 150$. Figures are all semi-diagrammatic.

cubical in shape, with a slight tendency to palisade arrangement. Depth of starch layer, $30\ \mu$ (Fig. 7).

R. bulbosus L. The slope is pronounced, directed upwards and

inwards, the angle being about 30 degrees. Depth of starch layer, $45\ \mu$ (Fig. 3).

R. Ficaria L. The slope is pronounced, with an angle of about 30 degrees, and is directed downwards and inwards. Depth of starch layer, $60\ \mu$ (Fig. 2).

R. flabellatus Desf. (*R. chaerophyllos* L.). This species can only claim to be British through occurring wild in the island of Jersey. It has a starch layer similar to that of *R. bulbosus*, but the slope may be a little less pronounced.

R. Flammula L. The starch cells have no slope, and are slightly elongated in the longitudinal direction parallel to the surface of the petal. There is no indication of palisade arrangement. Depth of starch layer, $38\ \mu$ (Fig. 8).

R. Lingua L. No slope. Starch cells similar to those of the previous petal. Depth of starch layer about the same.

R. ophioglossifolius Vill. This species is extremely rare in the British Isles, and we have only been able to procure a few specimens from one source. The results obtained suggest that the starch cells have no slope, but this requires confirmation when more material is available. Depth of starch layer, $22\ \mu$.

R. parviflorus L. This minute petal gave evidence of having starch cells with a pronounced slope directed upwards and inwards. The oblique character of these cells would appear to be even greater than in *R. Ficaria*, though directed in the opposite way. Depth of starch layer, $15\ \mu$ (Fig. 6).

R. repens L. The slope of the starch cells is fairly similar to that of *R. acris*, but perhaps a little more pronounced. The direction is the same. Depth of starch layer, $38\ \mu$ (Fig. 4).

R. sardous Crantz. (*R. hirsutus* Curtis.) The slope is not at all pronounced, though somewhat more apparent than in *R. arvensis*, and similarly directed. Depth of starch layer, $22\ \mu$ (Fig. 5).

R. sceleratus L. Slope is absent. The starch cells as viewed in longitudinal section are slightly elongated parallel to the surface of the petal and inclined to be rounded in contour. Depth of starch layer, $18\ \mu$ (Fig. 9).

COMMENTS.

From these observations it is obvious that the oblique growth of the starch cells is not an invariable accompaniment of gloss. Further it would seem that the intensity of the gloss is not exactly proportional to the slope of the starch cells. It is true that the petal of *R. Ficaria* with marked slope is very glossy, but not perhaps more so than that of *R. Flammula* with straight starch cells. It is just possible that slope may augment colour-depth though increasing the opacity of the petal. In the British

species, taken as a whole, the petals with little or no slope are less intensely yellow than those with pronounced slope, but this may be due actually to a less amount of pigment. From the figures given it is apparent that in petals more or less of the same size the depth of the starch layer increases with the slope of the cells; and this in itself would bring about greater colour-intensity in the petals with slope, the amount of pigment (which is restricted to the epidermal cells) remaining the same. *R. bulbosus*, for example, with more slope than *R. acris* or *R. repens*, has a deeper starch layer. *R. Lingua*, with the largest petal of all the British species of this genus, has a starch layer no deeper than that of *R. acris*. Probably the more the glossy area assumes the appearance of enamel, the deeper will be found the starch layer and the more inclined the starch cell.

With greater certainty one might draw the inference from these observations that the oblique growth of the starch cells is an advanced feature, and that primitively these cells were not so differentiated. The species *R. sceleratus*, *R. auricomus*, *R. Lingua*, and *R. Flammula*, with no slope, would appear to be less highly evolved than *R. acris*, *R. bulbosus*, and *R. repens* with decided slope. The petals of the former group have naked nectaries, or almost so, while the latter have these glands covered by marked scales. A naked nectary pit is presumably primitive to a covered one. Kumazawa in his morphological study of Japanese species of *Ranunculus* (2) considers *R. sceleratus* as probably one of the most primitive representatives of the genus.

The structure of the starch layer in *R. sardous*, *R. arvensis*, and *R. parviflorus* is not altogether as might have been expected. On account of these species having scales to their nectaries and tuberculate or prickly carpels, and being annuals, they might be regarded as more advanced than, for example, *R. acris*, and I was prepared to find their petals with pronounced sloping starch cells. *R. parviflorus* certainly appears to have such, but in the other two species, especially *R. arvensis*, the slope is only slight. These three species are generally put in the section *Echinella*, characterized by having tuberculate carpels. Quite possibly this style of achene may have evolved more than once independently in the genus. *R. ophioglossifolius* is interesting in this connexion. Its achenes are minutely tuberculate, and for this reason it has also been placed in the *Echinella* section; but present-day systematists are more inclined to regard it as belonging to the *Flammula* group of species. The structure of the starch layer as far as we have been able to examine it points that way.

The most striking outcome of this research on the structure of the starch layer is undoubtedly the opposite direction which the slope takes in the petal of *R. Ficaria* to that assumed in the other species. This is in keeping with the usual opinions held regarding the classification of the genus. *R. Ficaria* certainly in its general morphology stands apart, and

the early systematists recognized this so much as to place it in a separate genus, *Ficaria*. Perhaps a comparison might be drawn here between the occurrence of both right- and left-handed twining plants, and right- and left-spiralled snail shells. Of these opposites one form is usually much more common than the other. Dextrose twiners are more numerous than sinistrose, and dextral shells more so than sinistral. Probably a complete investigation of the genus *Ranunculus* would show that petals with the starch cells directed upwards and inwards are much more numerous than those with these cells sloping in the opposite way—at present only known in *R. Ficaria*.

The reason why these starch cells in development should assume this oblique form is obscure and so becomes a matter of speculation. Given a tendency for these cells to grow in length at right-angles to the surface of the petal, it is possible to imagine their being pulled out of the perpendicular into a slanting direction through the extension of the ordinary mesophyll cells lengthwise parallel to the surface. The study of the development of the petal of *R. Ficaria* suggests some such causal explanation (4, Figs. 4-7). But why the pull should be in one direction in *R. Ficaria* and in the opposite direction in the other species is not thereby explained.

Cells of this slanting type are distinctly unusual in plant tissues. They are known to occur occasionally in assimilating tissue. I am indebted to Professor Priestley for calling my attention to the literature. Pick (5) was the first to observe these cells, and thought that they assumed such a position in the mature leaf when illuminated by oblique light rays. Later investigators, including Haberlandt, do not subscribe to this view, and Liese (3) who has more recently studied the influence of light on the orientation of assimilating cells finds that the incidence of the rays may influence during development the direction taken by the palisade cells, but ceases to have any effect at maturity. These oblique palisade cells are, however, more of interest here as another example of an unusual form of tissue than as suggesting that the obliquity of the petal starch cells has any direct connexion with the incidence of the light rays. The petals during their development are not exposed to light, being well covered by the sepals.

From this study, as far as it has progressed, one receives the impression that the tendency in the genus has been to increase the opacity, and so the colour-intensity, of the petal by deepening the starch layer. This has been brought about by the cells assuming a palisade arrangement; but instead of their being developed perpendicular to the surface of the petal they have been pulled as it were during growth into an oblique direction.

SUMMARY.

1. The slanting of the starch cells of the petal is not universal in the British species of *Ranunculus*. The cells have—

- (a) no slope in *R. auricomus*, *R. Flammula*, *R. Lingua*, *R. sceleratus*, and probably *R. ophioglossifolius*.
- (b) A slight slope in *R. arvensis* and *R. sardous*.
- (c) A decided or pronounced slope in *R. acris*, *R. bulbosus*, *R. repens*, *R. flabellatus*, *R. parviflorus*, and *R. Ficaria*.

2. The direction of the slope in *R. Ficaria*, opposite to that in the other species with oblique starch cells, is emphasized and discussed.

3. Slope is regarded as a derived and not a primitive feature of these petals.

4. A causal explanation of the slope is tentatively suggested. It may be initiated when the starch cells tend in the course of evolution to assume a palisade character, i.e. to grow at right-angles to the surface of the petal. A pull on them during development by the extension of the adjacent cells lengthwise may cause the obliquity.

5. The tendency in the genus has probably been to increase the opacity, and so the colour intensity of the petal by deepening the starch layer.

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Investigation of the Effect of Age on Assimilation of Leaves.

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With six Figures in the Text.

CONTENTS.

	PAGE
I. INTRODUCTION	291
II. EXPERIMENTAL PROCEDURE	293
III. DATA AND DISCUSSION	293
A. Age-assimilation Relationship	294
(i) Morphological and Structural Characteristics	297
(ii) Water Content	297
(iii) Chlorophyll Content	298
(iv) Chemical Constitution of the Material	300
B. Development-assimilation Relationship in Leaves	303
IV. SUMMARY AND CONCLUSIONS	305
LITERATURE CITED	306

I. INTRODUCTION.

IN a previous study (11) on the limitations of Blackman's law of limiting factors and Harder's concept of relative minimum it has been emphasized that no definite relationship has so far been established between photosynthesis and the intensity of external factors *alone*. The internal changes that might be brought about in the photosynthetic mechanism by agencies other than the external factors are largely disregarded. For a clearer understanding of the process of assimilation, however, we have to fall back upon a more critical study of the nature, amount, and significance of photosynthesis with special reference to the internal factors and the

changes introduced into the mechanism by fluctuations in the relative concentration of the conditioning variables.

An investigation into the nature of the relatively obscure internal factors involves many difficulties as compared with a study of the external ones. As morphological and physiological changes take place with the development of the plant, so parallel changes in the internal complex of the photosynthetic machinery are but to be expected, as the external attributes are an expression of the plant response to both the external and the internal factors to which it is subject. An inquiry into the influence of age of the plant and the developmental stage of leaves on assimilation should, therefore, throw light on the nature of the internal factors concerned and the manner in which they interact to induce a specific change in the photosynthetic mechanism.

In such a study, however, it has to be borne in mind that all the other variables, except the one the influence of which is under consideration, must of necessity be kept above the limiting value and maintained constant throughout the period of investigation. In field experimentation, however, there are obvious difficulties in the maintenance of known conditions of external variables for a prolonged investigation such as the one in hand. Recourse has, therefore, to be made to laboratory experimentation where due consideration is given to the concentration of the external variables as it obtains in the field.

The present contribution aims at a study of the ontogenetic drifts in the rate of assimilation, with special reference to the relationship exhibited by the age of the plant and the developmental stage of individual leaves. Parallel experiments are performed with regard to the determination of optimum light intensity, CO_2 concentration, and temperature, and when the optimum values have been obtained the rate of assimilation at successive stages throughout the life-cycle of the plants is determined under such conditions. The assimilatory rate is subsequently analysed in terms of the internal factors, the chief among which being the morphological and structural characteristics, the hydration factor, the chlorophyll-content, and the chemical constitution of the material under investigation. An analysis of the assimilatory efficiency in terms of the intensity of these internal factors should thus show any correlation exhibited between the two sets of variables and facilitate the establishment of the relative importance of the internal factors in the synthesis of carbohydrates. In order to trace further the relationships, if any, between the end products of up-grade metabolism and the assimilation rate, the studies have been extended to crops of three different physiological groups. Sugar-cane is selected as a representative of the sugar group, possessing a relatively more simple carbohydrate constitution than either wheat or linseed, each representative of the more complex starchy-proteinaceous and fatty group respectively.

II. EXPERIMENTAL PROCEDURE.

The leaf materials selected for experimentation are obtained from well known crops of agricultural importance, viz., wheat (*var.* Pusa 4), linseed (*var.* 1150 C.), and sugar-cane (*var.* Reori) grown in the field under optimum conditions of nutrition and soil aeration at this experimental station.

The general procedure was to select representative young, mature, and old leaves on alternate days at regular intervals from a large population of green foliage collected from each of the crop plants mentioned above. To ensure similarity in the previous history of the leaves, they are placed in a beaker with the petioles dipping under water, and covered with an open bell jar near the north window of the fully ventilated laboratory which received sufficient diffuse light. After the lapse of about twenty-four hours the leaves are taken out, the petioles cut under water, the external moisture wiped off, the area traced, the fresh weight taken, and subsequently the rate of assimilation determined separately for each of the types mentioned.

The apparatus used consists of Blackman's commutator and clockwork arrangement with certain modifications to suit the experimental conditions described elsewhere (11). Barium hydroxide of a known strength is used as an absorbent for carbon dioxide and is titrated against standard hydrochloric acid with phenolphthalein as an indicator. Generally three to four hourly readings of apparent assimilation followed by two of respiration are recorded and the rate of real assimilation calculated to 100 sq. cm. of assimilating area per hour. To allow for the carbon dioxide absorbed by the baryta solution during the various operations, a washing factor has been included.

Observations are also recorded with regard to percentage water content, the chlorophyll content, the chemical constitution of the material, and the morphological and structural characteristics of the leaves at different stages of the life-cycle. The methods adopted for recording such observations are well known.

III. DATA AND DISCUSSION.

A survey of the literature on the subject indicates that our knowledge regarding age-assimilation characteristics in plants is extremely meagre, and the little work that has been done on this aspect of the problem does not relate to the age-developmental relationships of assimilation. Briggs (1-3) and Irving (9) studied the assimilatory activity and the factors which subsequently lead to the development of the photosynthetic machinery. Their observations being confined to the early stages, when the plants are not even well established in the soil, do not, as such, throw light on the question of age-assimilation relationships throughout the ontogeny. Willstätter and Stoll (12), on the other hand, experimented with leaves of

varying chlorophyll content and estimated the correlation between the amount of chlorophyll and the photosynthetic rate, the observations being based upon the survey of photosynthetic rate and chlorophyll content in leaves of varying developmental stages. Nowhere has the influence of the age of the plant on assimilation been directly studied throughout the life-cycle of the plants. In the present discussion, however, due attention has been paid to the influence on assimilation of age of the plant and developmental stage of individual leaves, and for convenience of interpretation they will be discussed under separate heads.

A. Age-assimilation Relationship.

Leaves of varying age—young, mature, and old—are gathered from average healthy plants of wheat, linseed, and sugar-cane at different stages in the life-cycle of the plants, and their rate of assimilation determined under known optimum external conditions. A glance at the assimilation rate of mature leaves (Tables I, II, III) at different stages of growth indicates that photosynthetic efficiency rises from the early stages up to a certain period of the life-cycle and subsequently shows a decline (Figs. 1, 2, and 3).

TABLE I.

Rate of Carbon Assimilation (Real Assimilation per 100 sq. cm. per hour) of Young, Mature, and Old Leaves of Wheat (Pusa 4) at Successive Stages of its Life-cycle.

CO₂ concentration 0.33–0.38 % : Temperature 27.5–28° C.
Illumination 375 C.P. (250 watt lamp at 4 cm. distance).

Date.	Days after germination.	Young.	Mature.	Old.	
		mg.	mg.	mg.	
21. 11. 31	7	0.80	—	—	
28. 11. 31	14	1.20	—	—	
5. 12. 31	21	1.60	—	—	
12. 12. 31	28	4.20	—	—	
19. 12. 31	35	5.70	5.80	—	
26. 12. 31	42	7.19	6.49	—	
2. 1. 32	49	9.54	8.64	—	
9. 1. 32	56	12.90	11.12	—	Initiation of floral primordia.
16. 1. 32	63	17.40	14.20	11.94	
23. 1. 32	70	21.90	18.62	15.00	Flowering.
30. 1. 32	77	15.30	13.55	10.45	
6. 2. 32	84	8.82	7.95	5.90	Yellowing begins.
13. 2. 32	91	—	7.14	5.57	
20. 2. 32	98	—	6.36	5.25	
27. 2. 32	105	—	3.78	4.10	
5. 3. 32	112	—	—	—	Leaves begin to dry up.

TABLE II.

Rate of Carbon Assimilation (Real Assimilation per 100 sq. cm. per hour) of Young, Mature, and Old Leaves of Linseed (115° C.) at Successive Stages of its Life-cycle.

CO₂ concentration 0.33-0.38 % : Temperature 27.5-28° C.
Illumination 375 C.P. (250 watt lamp at 4 cm. distance).

Date.	Days after germination.	Young	Mature.	Old.	
		mg.	mg.	mg.	
1. 12. 31	7	0.5	—	—	
8. 12. 31	14	1.07	—	—	
15. 12. 31	21	1.60	—	—	
22. 12. 31	28	4.60	—	—	
29. 12. 31	35	6.40	—	—	
5. 1. 32	42	7.90	—	—	
12. 1. 32	49	8.80	8.40	—	
19. 1. 32	56	9.60	9.20	—	Initiation of floral primordia.
26. 1. 32	63	12.60	8.90	—	
2. 2. 32	70	15.60	12.40	10.20	Flowering.
9. 2. 32	77	13.20	10.50	8.70	
16. 2. 32	84	10.80	8.60	7.20	
23. 2. 32	91	8.60	7.70	6.60	
2. 3. 32	98	6.40	6.80	5.90	
9. 3. 32	105	—	—	3.1	Drying of leaves begins.
16. 3. 32	112	—	—	—	

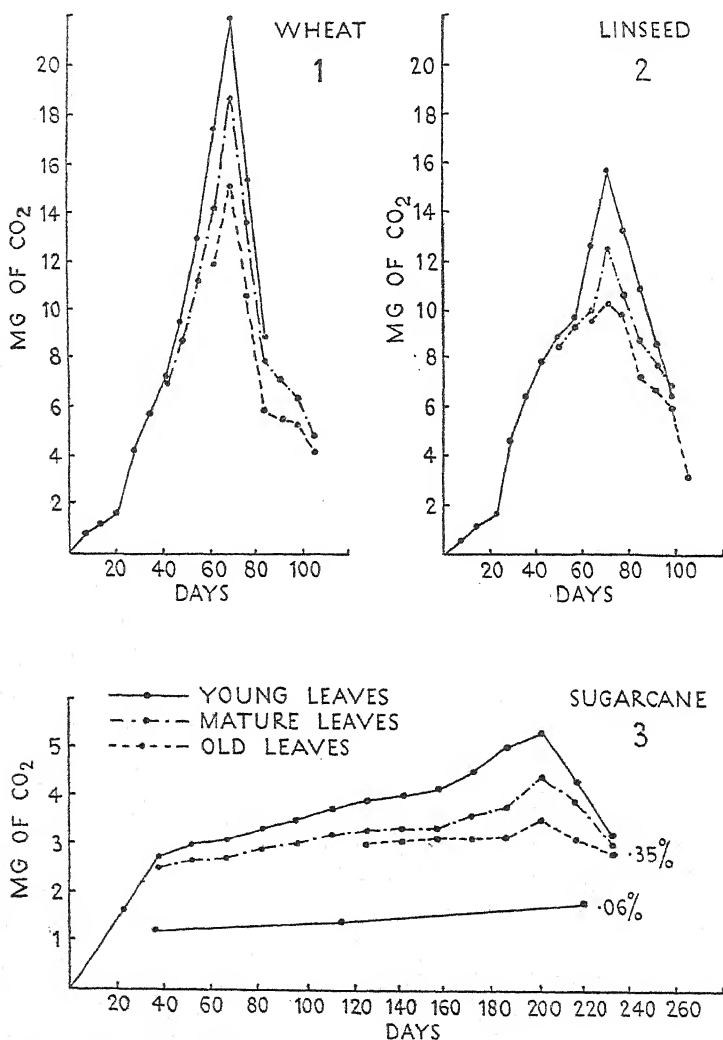
TABLE III.

Rate of Carbon Assimilation (Real Assimilation per 100 sq. cm. per hour) of Young, Mature, and Old Leaves of Sugar-cane (Reori) at Successive Stages of its Life-cycle.

CO₂ concentration 0.33-0.38 % ; Temperature 30.0° C.
Illumination 375 C.P. (250 watt lamp at 8 cm. distance).

Date.	Days after germination.	Young.	Mature.	Old.	
		mg.	mg.	mg.	
15. 4. 32	22	1.60	—	—	
1. 5. 32	37	2.70	2.50	—	
15. 5. 32	52	2.95	2.65	—	
1. 6. 32	67	3.05	2.70	—	
15. 6. 32	82	3.30	2.90	—	
1. 7. 32	97	3.50	3.00	—	
15. 7. 32	112	3.70	3.20	—	
1. 8. 32	127	3.90	3.25	3.00	
15. 8. 32	142	4.00	3.30	3.10	
1. 9. 32	157	4.10	3.30	3.10	
15. 9. 32	172	4.50	3.60	3.10	
1. 10. 32	187	5.00	3.80	3.15	
15. 10. 32	202	5.30	4.40	3.50	Maximum.
1. 11. 32	217	4.30	3.00	3.10	
15. 11. 32	232	3.20	3.00	2.85	Yellowing begins.

This characteristic age-assimilation relationship cannot be accounted for as being due to variations in the external factors, for they are maintained constant throughout the entire series of these investigations. We



FIGS. 1-3. Effect of age and developmental stage of leaves on photosynthesis. 1. Wheat. 2. Linseed. 3. Sugar-cane.

have, therefore, to fall back upon the possible variations introduced by a change in the internal factors with the age of the plant in order to explain the changes induced in the assimilation rate.

Such internal factors are many, among which, the morphological and structural characteristics of the leaf, the water content, the chlorophyll

content, and the chemical constitution of the material are but a few. The steady rise in the photosynthetic activity from week to week up to the stage of maturity clearly indicates that in the preceding week one of these factors at least was limiting and that with an increase in its intensity in the following period of observation the rate of assimilation rises. We shall discuss each of these in detail and finally conclude as to which is the prime factor and in what way the possible variations in the rate of photosynthesis is to be accounted for on the basis of the changes induced in such factors.

(i) *Morphological and Structural Characteristics.*

The very fact that assimilatory efficiency varies with the type of the leaf, the structural and anatomical features, the ecological characteristics and other induced variations, is a sufficient indication of the relation that exists between photosynthetic efficiency and the morphological and structural characteristics. In the present case, however, since the material supply comes from crop plants of pure strain grown under one ecological level of environment, it is not likely that the leaf tissue would undergo any serious anatomical and morphological changes with advance in age due to a variation in either the hereditary or environmental factors. Moreover, since the observations are recorded under controlled conditions of known external variables, the changes in the assimilation rate cannot as such be a result of environmental adaptation.

To throw more light on the question, the structural and morphological characteristics of the leaf materials under consideration have been studied at successive stages throughout the life-cycle of the plants. The structure of the epidermal tissue, the stomata, their arrangement and distribution, the characteristics of the palisade and the mesophyll cells were also taken into account. The structural characteristics of the leaves are found to remain practically the same at different stages of plant growth, the same typical anatomy being maintained throughout, although small variations in the stomatal aperture, the wall area, the chloroplast surface, &c., are to be noted. The variations are but negligible and hence cannot account for the rise in the photosynthetic activity. The rise should, therefore, be related to some other factor.

(ii) *Water Content.*

The investigations of Kreusler (10), Deherain and Maquenne (4), Iljin (8), and Dastur (5, 6) have shown that water supply is of importance to the photosynthetic process, not only on account of its indirect influence on the stomatal opening, but also in a very direct manner by entering into the chemical reactions of photosynthesis.

In nature, however, under normal conditions, water is usually present in excess, yet to ensure a sufficient supply to the experimental material the leaf base was kept dipping in water throughout the period of observation. To test the validity of this statement, the water content of the leaves is also determined as percentage of their fresh weight throughout the entire series of investigations.

A survey of the data (Table IV) brings into prominence the fact that the water content of the leaves remains more or less constant till the flowering period and later shows a continuous fall during the rest of the life-cycle (Fig. 4). This, when taken into consideration together with the assimilation rate, shows beyond doubt that the rapid rise in the rate of assimilation with advance in age of the plant is not the result of any disturbed water supply, but of some other factor.

(iii) *Chlorophyll Content.*

The establishment of a quantitative relationship between chlorophyll content and photosynthesis is exceedingly difficult on account of the impracticability of varying experimentally the concentration of chlorophyll. The method generally followed in such studies, therefore, has been to compare the assimilation rate in leaves of varying chlorophyll content. To this end, leaves showing varying degrees of greenness are gathered at successive stages of the life-cycle and their chlorophyll content determined by colorimetric methods simultaneously with their assimilation rate. This aspect of the problem has been worked out in detail in another paper of the series and only the data pertinent for the elucidation of the present point will be referred to.

The rate of assimilation of chlorophyll-rich (mature) and chlorophyll-poor (young) leaves are determined at different stages of plant growth. It is clear from Table I that a young wheat leaf 42 days old synthesizes 7.19 mg., while a mature one of the same age only 6.49 mg. under similar conditions. At a still later stage when the plant is 63 days old the readings for young and mature leaves are 17.4 and 14.2 mg. respectively. These values indicate that although mature or relatively old leaves contain a greater amount of chlorophyll than the younger ones at a particular stage of the life-cycle, they fail to show a proportionate increase in the photosynthetic rate. More or less similar results are obtained in cases of linseed and sugar-cane. The result is in agreement with the observations of Willstätter and Stoll (12), who found that even etiolated leaves after they had developed only a fraction of the chlorophyll content of the normal ones, might surpass the latter in the photosynthetic rate.

In this connexion the investigations of Emerson (7) on the photosynthetic relationship to different concentrations of chlorophyll in *Chlorella*

deserve special reference. The author has been able to establish a direct proportionality between the chlorophyll concentration and the photosynthetic rate. This disparity is not surprising, *Chlorella* being a simple

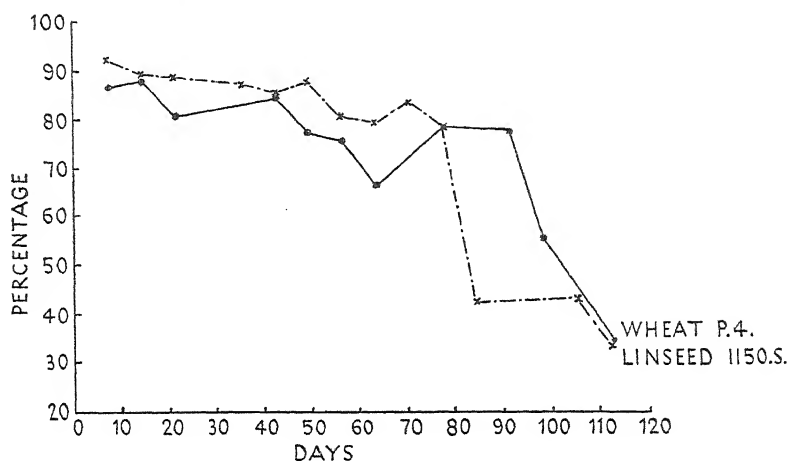


FIG. 4. Water content of leaves of wheat (P₄) and linseed (1150 S.).

TABLE IV.

Water Content of Leaves as Percentage of Fresh Weight of Wheat (Pusa 4) and Linseed (1150 C.) at Different Periods of its Life-cycle.

Age (days).	Wheat.	Linseed.	
7	86.3	92.4	
14	87.7	89.4	
21	80.9	88.4	
28	—	—	
35	—	87.7	
42	84.2	85.5	
49	77.1	87.7	
56	75.9	80.3	
63	66.1	79.6	
70	76.3	83.9	Flowering.
77	78.4	78.6	
84	—	42.4	
91	77.6	—	
98	56.3	—	
105	—	43.3	
112	34.8	33.3	
119	7.4	—	

unicellular organism has the simplest mechanical arrangement for the CO₂ absorption. With multicellular leaves, however, the mechanism is considerably complicated by the presence of stomata. The uniform diffusion of carbon dioxide to all the chloroplasts within the green tissue becomes still more difficult owing to the overlapping of the green corpuscles at different depths. The maintenance of a practically uniform intensity of

light throughout the deeper regions also involves practical difficulty; we may thus expect that considerable changes may be brought about in the rate of assimilation by the varying degrees of resistance met with in the diffusion and photochemical phases of the mechanism.

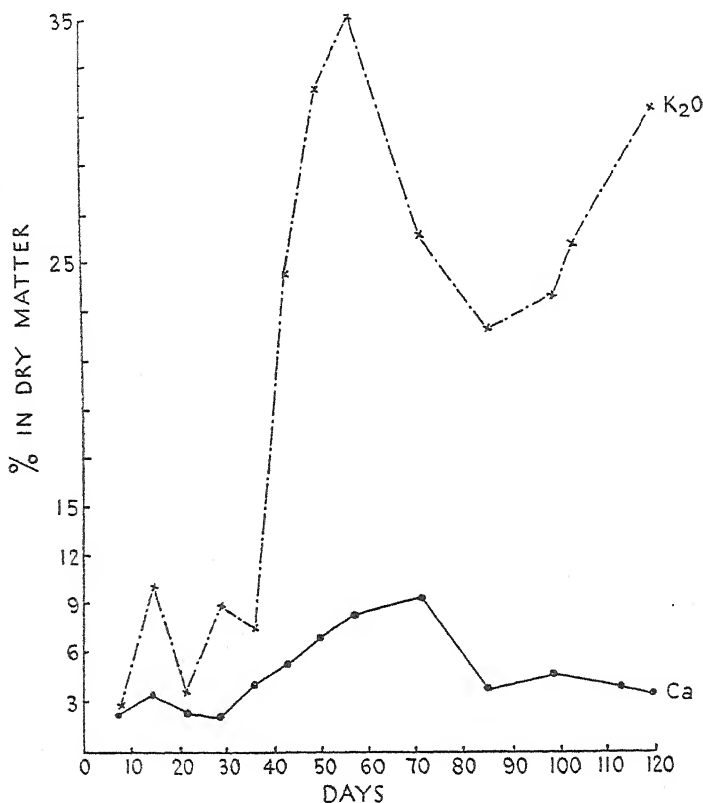


FIG. 5. Inorganic constituents, wheat (P 4).

We thus naturally arrive at a conclusion that the rise in the assimilation rate can neither be accounted for on the basis of morphological and structural changes, nor can they be explained as due to variation in the hydration factor or the chlorophyll content. There are other factors probably which have a greater effect on the process. We now proceed to examine more critically the assimilation rate in terms of the chemical data of the material under investigation.

(iv) *Chemical Constitution of the Material.*

Detailed chemical analyses of the leaf material at different stages of growth have been conducted with special reference to carbohydrates, celluloses, proteins, fats, and ash. Of the various chemical constituents of the

leaf material the most striking correlation is shown by the calcium and potassium content. The quantity of calcium in wheat (Fig. 5) rises from the early stages up to a period of seventy days and afterwards shows a decline similar to the rate of assimilation. The requirement of potassium

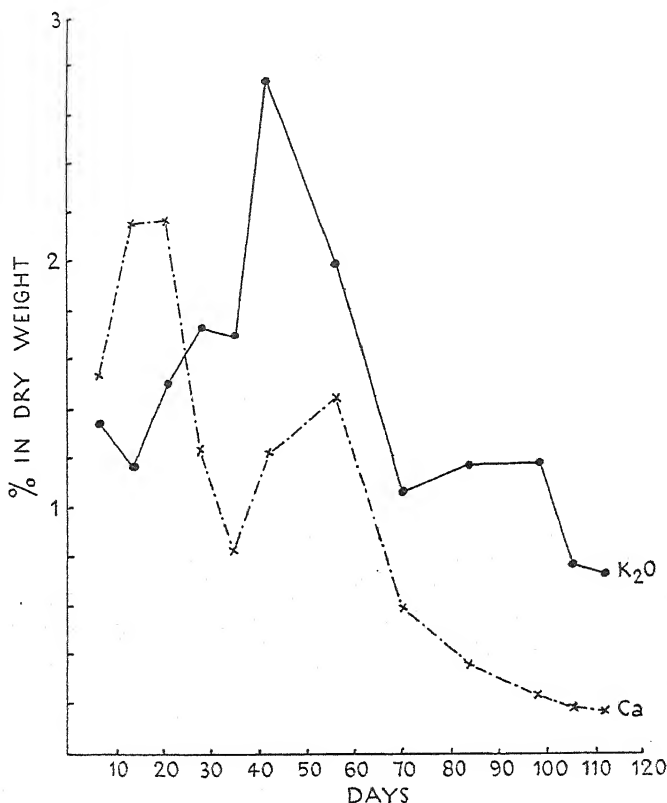


FIG. 6. Inorganic constituents, linseed (1150 C).

and calcium in linseed (Fig. 6) also exhibits positive correlation with the assimilation rate. This close similarity between the photosynthetic curve and that for calcium and for potassium, even in plants so different as wheat and linseed, suggests an important physiological connexion between these elements and the rate of synthesis of carbohydrates, whether these elements play a part in reducing the carbon dioxide to formaldehyde or change the velocity of the reaction by removing the products of synthesis as soon as they are formed, or whether they neutralize the harmful effect of toxic by-products of carbohydrate and protein synthesis and thus indirectly increase the rate of synthesis, is of course still uncertain.

The measurably increased rate of the synthesis of organic materials

during the middle portion of the life-cycle may well be attributed to increased catalytic action brought about by potassium and calcium.

With the second portion of the age-assimilation curves of wheat (Fig. 1) it is found that after the maximum has been reached, a characteristic decline phase becomes explicit, for within a period of seven days the values fall from 18.62 to 13.55, i.e. there is a 25 per cent. decline in activity. That chlorophyll content can account for this is unlikely, for the leaves are found to possess more or less the same chlorophyll content.

A reference to Figs. 4, 5, and 6, however, indicates that the water, as well as the potassium and calcium content of the leaf, shows an abrupt decline after the maximum period of assimilation, and it is just possible that a sudden change in two of the most important internal conditions may affect the photosynthetic mechanism. With advance in age the supply becomes more and more limited, as shown by a rapid decline in the curves of water content on the one hand and potassium and calcium on the other. In consequence the rate of assimilation, too, undergoes severe limitations leading to a continuous decline in the curves towards the senescent stage. More or less similar inferences are to be drawn from the experiments performed with linseed and sugar-cane, with slight differences in the magnitude of photosynthesis and also the location of the maximal hump.

We have discussed the effect of ageing on assimilation under optimum conditions of external factors and shown how the values of assimilation increase for a certain period of the life-cycle and later show a decline. To test whether such behaviour is also observed under natural conditions, a series of other experiments have been performed.

In nature, especially in these subtropical regions, sunlight is always above the limiting value except in the rainy season and during the early and late hours of the day. The air temperature is also found to be sufficiently high and at times it becomes fatal to the leaves. Both these factors thus remain at fairly high optimum values. So far as the carbon-dioxide concentration is concerned, practically no data exist. Experiments were therefore performed to determine the CO_2 concentration of the atmosphere in fields at different depths. Only a representative case of a sugar-cane field where carbon-dioxide percentage is measured at a height of two feet from soil-level is cited below.

A survey of the data (Table V) indicates that the concentration of CO_2 in the field is always above the normal percentage of the atmosphere. It varies from hour to hour, reaching a maximum towards the later periods in the night and a minimum in the afternoon. The maximum values in the night are due to the respiration of the vegetable materials in the surroundings, while as the day dawns and light is available, the extra CO_2 is removed and once again the readings show a decline. The mean percentage is 0.061 only.

TABLE V.

Variations during Twenty-four Hours in the Carbon-dioxide Content of the Atmosphere of Sugar-cane Fields, taken 2 ft. above Soil-level.

Time.	Percentage.
7 A.M.—10 A.M.	0.051
10 A.M.—1 P.M.	0.051
1 P.M.—4 P.M.	0.048
4 P.M.—7 P.M.	0.048
7 P.M.—10 P.M.	0.048
10 P.M.—1 A.M.	0.061
1 A.M.—4 A.M.	0.0916
4 A.M.—7 A.M.	0.0916

Mean 0.061.

TABLE VI.

Rate of Carbon Assimilation (Real Assimilation per 100 sq. cm. per hour) of Mature Leaves of Sugar-cane (Reori) at Successive Stages of its Life-cycle.

CO₂ concentration 0.06 %; Temperature 30–30.1° C.
Illumination 375 C.P. (250 watt lamp at 4 cm. distance).

Date.	Days after germination.	Mature leaves. mg.
1. 5. 32	37	1.2
18. 7. 32	115	1.4
4. 11. 32	220	1.8

Using such a concentration of carbon dioxide, the temperature and light being the same as in the experiments already cited, readings of assimilation rate of mature leaves are taken. To take a representative case, it is seen (Table VI) that the assimilation rate remains more or less constant throughout, only showing a slight increase towards the maximum period of assimilation, i.e., 220 days after germination. This more or less stationery rate of photosynthesis is to be accounted for on the basis of limiting factors, the pace of the reaction being controlled by the low concentration of carbon dioxide. The internal factors, although favourable to a higher rate of assimilation under high concentrations, are not able to exhibit their maximum efficiency in a low CO₂ concentration. Thus the characteristic rise in the assimilation curve till the period of maturation and the subsequent decline are not to be observed under natural conditions of the atmosphere where CO₂ is the limiting factor.

B. Development-assimilation Relationship in Leaves.

Since the age of the plant brings about changes in the assimilation rate it is probable that the developmental stage of the individual leaf

might also alter the assimilation rate. To test this a series of experiments have been conducted with wheat, linseed, and sugar-cane.

The assimilation rate of leaves of varying developmental stage has to be determined at one and the same stage of the life-cycle, for if the age of the plant is also varying, the variations, if any, would be a resultant of the effect of the age of the plant as well as the developmental stage of individual leaves. Leaves at various developmental stages have been gathered at the same stage of the life-cycle in wheat, linseed, and sugar-cane, and their assimilatory efficiency determined under external optimal conditions as in the previous cases.

As representatives of the total population of leaves of varying development, the data relating to the young, mature, and old leaves are chosen for illustration. From the values of their assimilation rate (Tables I, II, III) it is apparent that the young leaves show a greater rate of assimilation than the more developed or older ones. Without exception, at every stage in the life-cycle of all the three crops, the same phenomenon is marked, though the limits of variation are considerably altered on account of the simultaneous changes introduced by the age of the plant.

The variations noted above may be due to the structural peculiarity of the leaves, especially to the thickness of the palisade cells, changes in the respiratory index of different leaves, or to other causes. But a review of the data shows that the low rate of assimilation of older leaves as compared with that of younger ones is due not only to a lower rate of respiration, but also to the decrease of apparent assimilation. Older leaves show low apparent assimilation, even though they have undoubtedly higher chlorophyll content. The cause of the decreasing rate, in spite of this higher content, is most probably the thickness of the older leaves. In older leaves the thickness of the palisade cells may reduce the CO_2 reaching the deep-seated chloroplasts which, therefore, do not function as efficiently as in younger leaves.

Mention may be made in this connexion of the work of Willstätter and Stoll (12), who demonstrated that light and dark green leaves of *Acer pseudoplatanus* have a chlorophyll content respectively of 8.3 and 40.0 mg. per 10 gm. of fresh weight, whereas the assimilation rate in the second increased by approximately one and a half times only.

A review of the data for all the three crops (Tables I, II, III) further shows that such differences in the rate of assimilation of young, mature, and old leaves is evident only during the middle portion of the life-cycle of the plants. Before and after a particular age, 35 and 98 days respectively to quote the case of wheat, the different types of leaves show practically the same photosynthetic rate. During the early stages, therefore, they are all of one type, viz. young or juvenile, while towards the end of the life-cycle they may well be grouped as old or senescent. Thus the mechanical

classification of leaves into different groups—young, mature, and old—either in the early stages or during the senescent phase, may not in any real sense represent the order of their photosynthetic efficiency, since the leaves of different developmental stages during these periods show practically the same rate of assimilation. The morphological and chronological classification of leaves, therefore, is no criterion of their physiological activity.

The above remarks on the effect of age of the plant, and the developmental stage of leaves on assimilation, lead one to the conclusion that of the many factors contributing towards senescence in plants, a decline in photosynthetic activity is one. The gradient of age-assimilation curves for the crops under consideration is marked by a number of phases, prominent among which are the young, or juvenile, the mature or adolescent, and the old or senescent stage. These three periods in the life-cycle of the crops, based as they are on the activity of assimilation, clearly show characteristic optimal activity in the young leaves, followed by a rate of slightly decreased activity in mature ones. In the case of old foliage the rate of photosynthesis in all the cases reaches a period of lull showing a gradual fall, to be succeeded by a sharp decline during the period of yellowing and drying of the leaves.

The causes of the period of increased activity, the lull, and finally old age in the plants are in question. The causal factors leading to the rise and fall in the rate of assimilation have been discussed. It has been pointed out that, among others, the percentage water content of leaves, the amount and surface of the chlorophyll, the nature and quantity of such substances as calcium and potassium are mainly responsible for regulating the photosynthetic activity of the plant. The increase in photosynthesis during the young stage appears to have a direct relation with calcium and potassium, while a decrease in the absorption of these elements towards the latter period may be associated with the period of inactivation. The high or low rate of assimilation may possibly be related to the activity of these elements as catalytic agents.

IV. SUMMARY AND CONCLUSIONS.

Determinations are made of the rate of photosynthesis of the entire population of leaves segregated into classes, as young or juvenile, mature or adolescent, and old or senescent leaves, at successive stages throughout the life-cycle of wheat, linseed, and sugar-cane. The rate of assimilation is determined under optimal concentration of light, CO_2 , temperature and water, so that no limitation should arise from the external variables. The changes in the rate of photosynthesis therefore becomes a function of the internal factors. Analysis of the data relating to the internal factors, such

as the water content of the leaves, the chlorophyll content, the presence of possible inorganic catalysts, as also morphological and structural characteristics of leaves reveal interesting features.

The following conclusions are the outcome of the present investigation :

The age-assimilation data for mature leaves in all the three crops under *optimal* conditions yield a characteristic curve beginning with a low value while the plants are young, attaining a maximum at a time when the plants show maximum growth and maturity, and slowing down with age till assimilation ceases towards the senescent stage of the crop.

Under a reduced concentration of carbon dioxide, equivalent to that of the atmosphere, this characteristic curve merges into a level phase such that the initial values are maintained throughout the life-cycle, indicating that under natural conditions, carbon dioxide limits assimilation and, therefore, the growth of crops.

Besides the water factor and the chlorophyll content of the leaves, the presence of presumed catalysts such as calcium and potassium seems to govern the intensity of assimilation. Where other factors are not limiting, the greater the amount of these, the greater the intensity of assimilation.

At any stage in the life-cycle a change in the developmental stage of leaves is characterized by a variation in their assimilation rate. The young and mature leaves show a high and a medium efficiency respectively, while the old ones exhibit a low photosynthetic rate.

The chronological and morphological classification of leaves is no criterion of their assimilatory activity, for before and after a definite age of the crops all the leaves are more or less of one physiological type.

The age and developmental stage of leaves is an important internal factor controlling the intensity of assimilation as also its mechanism.

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The Interaction of Factors in the Growth of Lemna.

VI. An Analysis of the Influence of Light Intensity and Temperature on the Assimilation Rate and the Rate of Frond Multiplication.

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With Fifteen Figures in the Text.

CONTENTS.

	PAGE
I. INTRODUCTION	309
II. METHODS AND TECHNIQUE	310
III. DATA AND STATISTICAL ANALYSIS	316
IV. DISCUSSION OF DATA	325
V. SUMMARY	334
LITERATURE CITED	336

I. INTRODUCTION.

THE duckweed, *Lemna minor*, has been used for physiological experiments in this laboratory since 1927. It has many advantages for such work: it multiplies rapidly in inorganic water cultures, and colonies may be grown under closely controlled conditions of the environment. Moreover, since there is no translocation away from the frond, very little differentiation of tissues, and according to the authors' observations the stomata are non-functional, many of the complexities which attend the physiology of higher plants are avoided, and the analysis of the effects of environmental factors on the growth processes is simplified.

Experiments already published in this series have shown, somewhat cursorily, how the rate of multiplication of fronds is affected by the length of daily illumination (2), the presence of minute quantities of organic matter (3), and the temperature and intensity of light (10). In the present

paper fuller data are given for the influence of light intensity and temperature upon:

- (i) The relative rate of multiplication of fronds (relative growth rate);
- (ii) the net assimilation rate;
- (iii) the frond area;
- (iv) the frond weight.

It has been established by several workers (9, 11) that the law of limiting factors as put forward by F. F. Blackman in 1905 (4) does not apply rigidly to the effects of certain environmental factors upon assimilation rate, and it is equally clear that the law will not hold rigidly for relative growth rate. It has become necessary therefore to investigate with greater accuracy the interaction of environmental factors on these processes, with a view to replacing the principle of limiting factors by something more comprehensive. Several workers have already collected data on these lines, but there seems to be no information as to the interaction of light and temperature on the growth processes over a wide range of conditions. It is the aim of the present work to provide data on this subject, together with a formal analysis of them.

A preliminary series of experiments was carried out by the senior author in 1928-9 (10). It was found that great care was necessary in the treatment of colonies of *Lemna* before each experiment, since their behaviour during an experiment reflects their immediate previous history. The results of these preliminary experiments together with those of Hicks (10) are inconclusive, since the data cannot be subjected to adequate statistical analysis.

In the years 1932-4 a series of experiments was carried out under more critical conditions, over a wider range of temperatures and light intensities, and in such a way that any variance due to the occasion of an experiment could be separated from the total variance of the data. In this way it has been possible to eliminate any error due to seasonal variation in the response of colonies to the experimental conditions. The following pages are devoted to a description of the technique employed and an analysis and discussion of the data obtained from these experiments.

II. METHODS AND TECHNIQUE.

A growth chamber was designed in which eight light intensities and two temperatures could be maintained simultaneously, so that colonies of *Lemna* could be grown under sixteen combinations of light intensity and temperature at one and the same time. This has an advantage over the apparatus previously used (2), in which only four light intensities and one temperature were obtainable together. It was found unnecessary, however, to aerate the solutions as was done in previous experiments. Accordingly

colonies were grown in ordinary beakers in which the solution was changed every twenty-four hours.

Description of apparatus. A labelled diagram of the elevation of the apparatus is given in Fig. 1. Two rectangular zinc water-baths, each 6 ft. long by 1 ft. wide by 10 in. deep, are placed side by side on a wooden base-board. The temperature in each bath is controlled by a mercury thermostat, each thermostat operating four electric heaters through a relay. Since each tank has a capacity of thirty gallons and is effectively insulated by a wooden box, variations in temperature of more than 0.25°C . are rare. Within each tank is a stirrer which effectively prevents inequalities of temperature. Each tank has a zinc lid which is perforated by sixteen holes, each 9 cm. in diameter, in two rows of eight, in which are placed the beakers containing the experimental colonies. Over the top of the tanks is fixed a wooden box painted white inside, and having as its floor the lids of the tanks. The box is divided transversely by removable wooden partitions into eight light chambers, each light chamber covering two holes in each tank. Thus, in the chambers, along the length of the box, eight light intensities can be maintained, and beneath each chamber two different temperatures.

The colonies of *Lemna* are contained in 400 c.c. beakers, which are suspended by their rims in the holes already described, and immersed in the water (Fig. 1). By opening two doors in the front of the box the eight light chambers are exposed, and colonies may be removed or examined at will.

From the roof of each light chamber hang two electric lamps. The lamps are on brass tubes half an inch in diameter, which pass through holes in the roof of the light chamber. The tubes are kept in position by collars with set-screws, by means of which it is possible to adjust the height of the lamps above the colonies, and thus to adjust the light intensities. The heights of the lamps in each chamber are so arranged that the colonies at both temperatures are subjected to the same light intensity. The heating effect of the lamps is minimized by enclosing them in glass water-jackets through which cold water is run continuously.

Although the apparatus as designed will hold thirty-two colonies, i.e. duplicate colonies under each condition, it was found impracticable to cope with the sampling of such large populations. Accordingly, in most of the experiments one colony only was grown at each combination of light and temperature.

The light intensity was measured by means of a direct reading photo-electric illuminometer calibrated in foot-candles, supplied by the Weston-Electrical Instrument Corporation. A shallow round tray was made which fitted freely into any of the holes in the tank lid; in this the photo-electric cell was placed when light intensity measurements were made. The depth

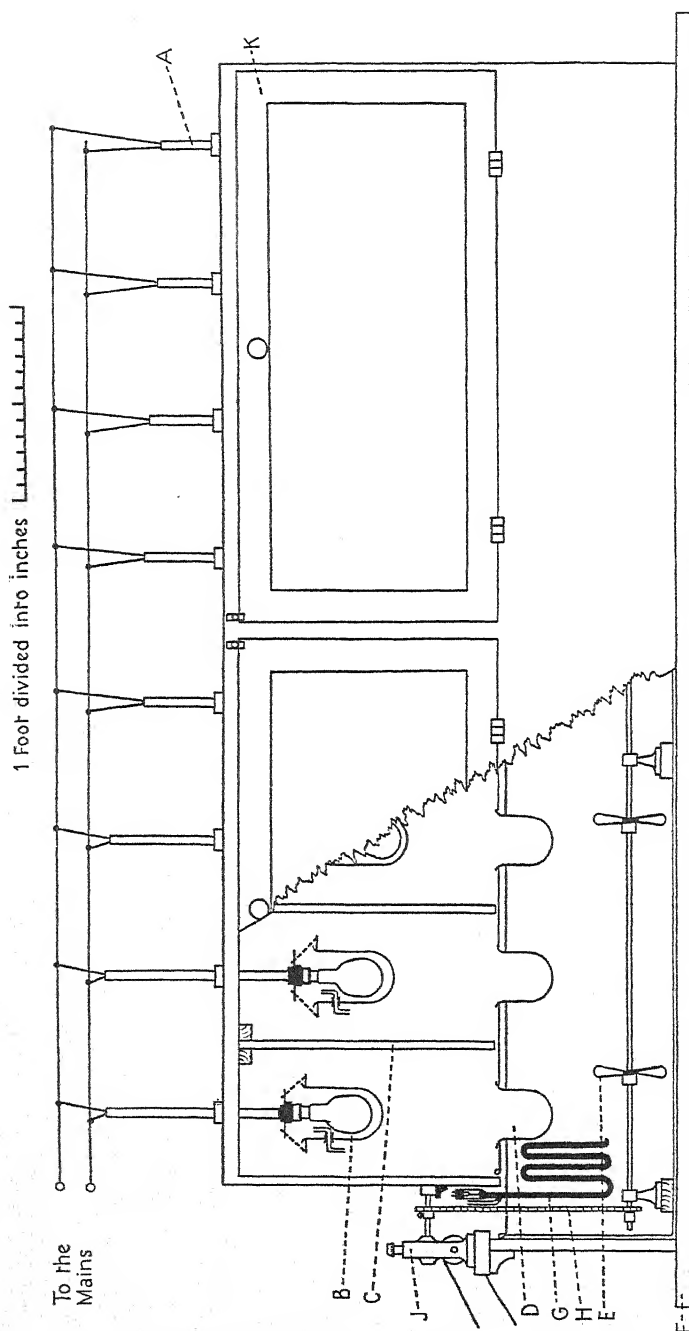


FIG. 1. Growth chamber used for cultures of *Lemna* described in this paper. A, brass tube supporting lamp; B, glass water jacket surrounding lamp; C, partition between light chambers; D, containers in which colontes are grown; E, stirring paddle; F, wooden baseboard; G, thermostat; H, chain driving stirrer; J, reduction gear for stirrer; K, door of chamber.

of the tray was such that the sensitive surface of the cell was brought to the level of the culture solution in the beakers. During the course of each experiment the light intensities were tested about three times a week, and when necessary the heights of the lamps were adjusted accordingly. This was occasionally found to be necessary, as the light emission of electric lamps falls off gradually during their life. Transient fluctuations of light intensity of about 10 per cent. were unavoidable owing to variations in the voltage of the electricity supply.

Dry weight technique. This is based on the method described in an earlier paper (12), with alterations due to the fact that it was possible in the present experiments to take larger samples (50 to 100 fronds). Drying was carried out in small glazed porcelain crucibles weighing approximately 1 gm. each. These were dried thoroughly by exposure to a temperature of 95° C. for forty minutes *in vacuo*, in the apparatus described by Su and Ashby. They were then weighed and the samples, taken as described on p. 314, were packed loosely into them. The process of drying was then repeated upon the crucibles containing the samples of fronds, which were then removed one at a time from the desiccator and weighed immediately upon a Bunge micro-balance. The weight of the crucible being known already, a weighing could be completed within one minute. Although the crucibles were not kept covered, the increase in weight due to absorption of water during the period of weighing was always less than 0.01 mg.

Area technique. For the measurement of frond area the technique formerly used (1) was found to be so laborious that all the samples could not be measured on the day on which they were taken. For this reason a new method employing a photo-electric cell was devised, which was found to increase the facility of measurement considerably. The apparatus is illustrated in Fig. 2.

A beam of light passes upwards through a condenser lens (*a*) focused on the lens of a camera (*b*) placed vertically above it. In the back of the camera is fixed a Westinghouse photo-electric cell (*c*). This cell has an evenly sensitive circular surface, 25 mm. in diameter. It is connected to a mirror galvanometer, the deflection of which is proportional to the total amount of light falling on the cell. Immediately above the condenser is a glass-bottomed cell (*d*) which is filled to the brim with water. The camera is adjusted so that the image of the water surface is focused on the photo-electric cell and falls entirely within its limits. The light intensity is adjusted in such a way that the galvanometer gives a maximum deflection. If *Lemna* fronds are floated on the water surface they will cut off an amount of light proportional to their area. This will result in a change of the deflection of the galvanometer proportional to the amount of light cut off. The change in galvanometer deflection is therefore a measure of the area of the sample.

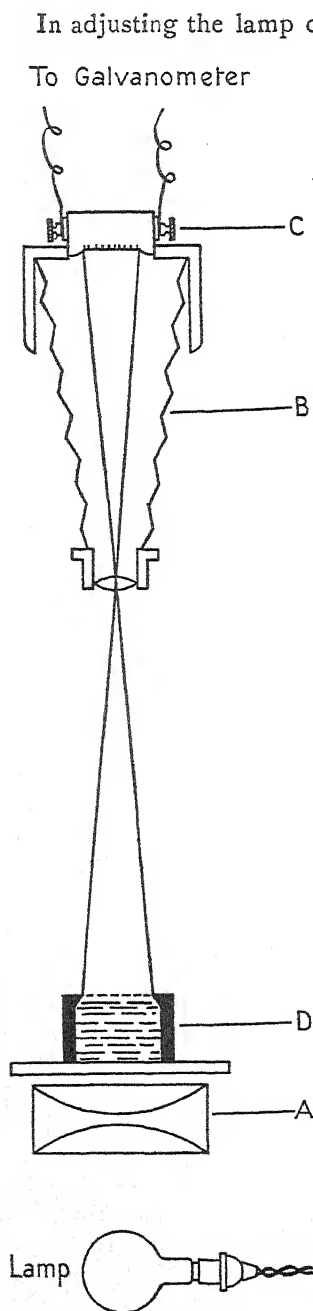


FIG. 2. Diagram of photo-electric apparatus for the measurement of frond area. For description and explanation of lettering see text, p. 313.

In adjusting the lamp care is taken to ensure that the image of the water-cell is evenly illuminated. This was checked in practice by moving various sized colonies of *Lemna* over the surface of the water to different parts of the cell. It was found that the reading remained unchanged however the *Lemna* was distributed on the surface. The apparatus was calibrated, using standard areas of cardboard and colonies whose area was determined by the original enlarger method (1). It will be seen from Fig. 3 that the calibration graph is a straight line. A certain amount of light passes through the fronds. This, however, is completely diffused so that only a negligible proportion reaches the lens of the camera. The image of the fronds appears in fact quite black.

Procedure. Before discussing the procedure followed, the results of certain preliminary tests are given: (i) In order to test the precision with which any combination of light and temperature could be produced, the light intensities were adjusted to 350 foot candles over each of eight colonies of *Lemna* distributed in four light chambers. The eight colonies were grown for ten days at a constant temperature of 25°C. and were counted daily for the last eight days of the period. The growth rates of the colonies were then calculated, and it was found that none differed significantly from their mean, and the percentage standard deviation was 1.88 per cent. (ii) In order to find the approximate sampling error attached to the area estimations, eleven samples were taken from an apparently uniform population of *Lemna* and their area was measured. The percentage standard deviation was 6.17 per cent. In a similar test for the sampling error of dry weight estimations five samples were taken of various sizes, and the percentage standard

deviation was found to be 6.5 per cent. It is evident that the greater part of these deviations is due to biological variation of the material rather than to errors inherent in the apparatus.

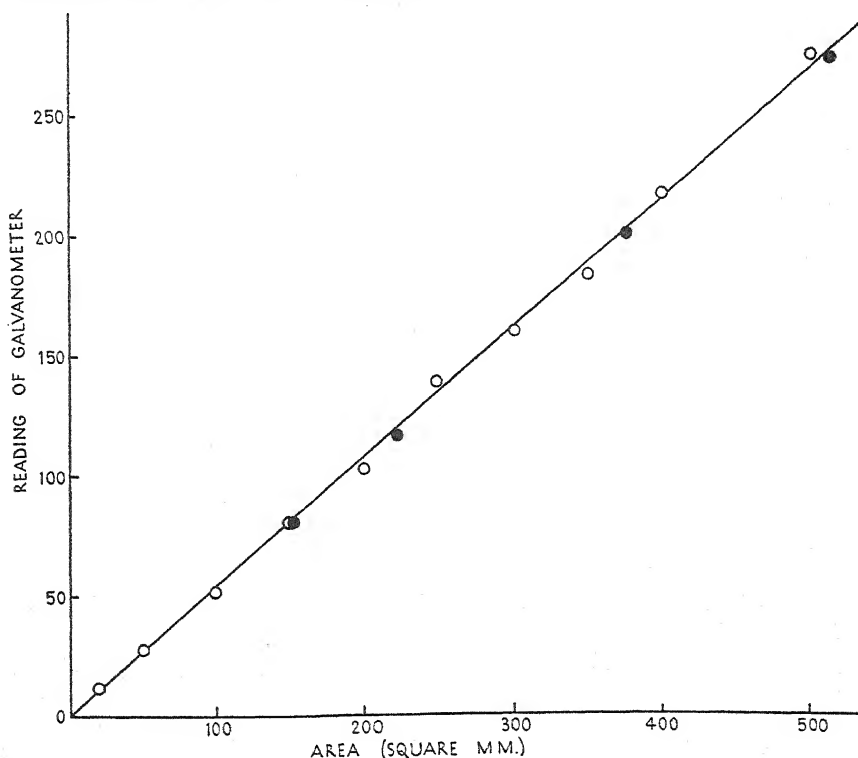


FIG. 3. Calibration graph for the apparatus for measurement of frond area. The circles represent values obtained in the initial calibration with pieces of paper of known area; the black points represent values obtained, using colonies of *Lemna* whose area was afterwards determined by the enlarger method (see Ashby, Bolas, and Henderson (1)).

The stock of *Lemna* is a clone from one frond originally brought from Kew in 1931. Owing to the fact that the behaviour of *Lemna* under experimental conditions is influenced by the previous treatment, great care was taken to maintain the colonies in a controlled environment between the experiments. They were kept in the same culture solution as was used for the experiments; the light intensity was 350 foot candles, and the temperature 18° to 20° C.

The culture solution was the same as that used in earlier experiments,¹ except that it was made up with tap water instead of 'glass-distilled' water. This was more practicable since about 50 litres a week are required, and

¹ $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ 0.1008 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2465 gm.; KNO_3 0.8088 gm.; FeCl_3 0.0016 gm.; water, one litre.

satisfactory since the composition of tap water varies very little.¹ Owing to the presence of alkali in the tap water the pH of the solution is too high. It was adjusted to pH 4.8 by the addition of about 14 c.c. normal sulphuric acid to fifty litres of culture solution.

At the beginning of an experiment some seventy fronds were placed in culture solution in each of the sixteen beakers, the eight light intensities and two temperatures having been adjusted to the values required for the experiment. The solution was changed daily in each of the beakers, but no counts of frond number were made or samples taken for about five days, after which period the multiplication rate becomes exponential. At the end of five days the number of fronds in each colony was reduced to 100, the surplus being taken as samples for the determination of dry weight and area per frond. The experiment was then continued, with daily counting of frond number and changing of solution for about twelve days. Twice a week (in some cases more frequently) the number of fronds in each colony was reduced to 100, the surplus thus removed being used for dry weight and area determinations. From the daily frond numbers obtained the number of fronds which *would have been* present had there been no reduction was calculated by proportion.

III. DATA AND STATISTICAL ANALYSIS.

In this section are set out the data obtained in the experiments, together with statistical tests of their significance. Discussion of these data is deferred to Section IV, where the results of these analyses are summarized as graphs.

Experiments were performed at five temperatures: 10°, 18°, 21°, 24°, and 29° C. At each temperature colonies of *Lemna* were grown under eight light intensities: 80, 150, 350, 500, 750, 900, 1,100, 1,600 foot candles. Data have been collected, therefore, for the behaviour of colonies under forty conditions of light intensity and temperature. The experiments at 18°, 24°, and 29° were duplicated at different times of the year, in order to discover whether there was any seasonal drift in growth rate.² Each experiment was continued for seventeen to twenty days. Data for frond number, area, and dry weight were collected according to the methods described in Section II. A preliminary set of experiments was carried out in 1933, at light intensities from 100 to 1,500 foot candles, and temperatures from 21° to 35° C. These experiments cannot be analysed together with the present experiments, since the combinations of light and temperature used were different. The data conform to those collected in 1934,

¹ Water distilled from copper was not used, since the colloidal copper inhibits the growth of *Lemna*.

² The dates of the experiments were as follows:—experiments at 10° and 18° in January, 1934; at 18° and 21° in March 1934; at 24° and 29° in April 1934; and at 24° and 29° in June 1934.

and one experiment is presented in condensed form with some remarks upon it on page 324.

(i) *Data of frond number.* The frond is the morphological unit in *Lemna*, and each frond is the product of one primordium. The relative

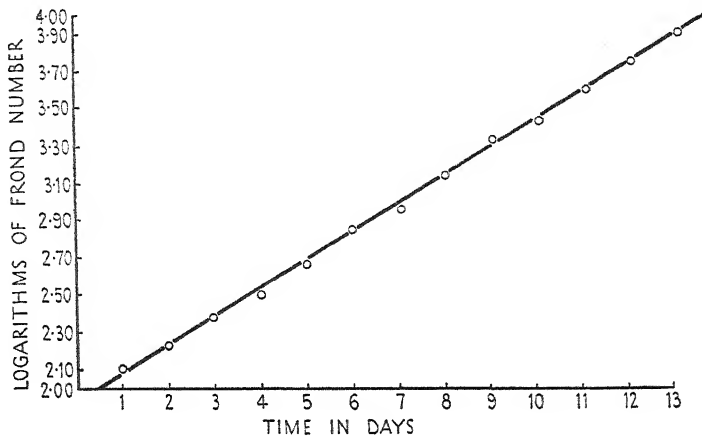


FIG. 4. Logarithms of frond number plotted against time in days. The figures are taken from a typical experiment at 24°C. and 500 foot candles. The straight line drawn through the points corresponds to the equation $y = 0.152x + 1.935$.

TABLE I.

Comparison of Frond Number observed and calculated from the Regression Equation $y = 0.152x + 1.935$. Data from Experiment at 24°C. and 500 foot candles.

Day.	Frond numbers.	
	Observed.	Calculated.
0	100	86
1	127	122
2	171	173
3	233	245
4	323	368
5	452	493
6	654	699
7	918	990
8	1406	1404
9	2150	2137
10	2800	2822
11	4140	4001
12	5760	5672
13	8250	8042

rate of frond multiplication is therefore a useful measure of the relative growth rate of a colony. Under all combinations of conditions the relative rate of increase of frond number was constant for the duration of the experiment, so that the logarithms of frond number plotted against time

fall conformably along straight lines, whose slopes give a measure of the relative multiplication rate (Fig. 4). Since these values naturally do not fall *exactly* on straight lines, the slopes cannot be calculated accurately unless lines of closest fit are obtained by the method of least squares. Such lines have accordingly been found for all the data. The lines were calculated from the general formula $y = ax + b$, and the constant 'a' (the regression coefficient) for each calculated line measures its slope, i.e. the relative multiplication rate of the colony. Each regression coefficient is calculated from a dozen or more daily observations of frond number. That the calculated coefficients are a satisfactory measure of the observed rates of development will be seen from an inspection of Table I.

The regression coefficients, i.e. the values for relative rate of multiplication, are assembled in Table II.

TABLE II.

Regression Coefficients of Lines of Closest Fit of the Curves of log. of Frond Number plotted against Time.

Temperature	10° C.	18° C.	18° C.	21° C.	24° C.	24° C.	29° C.	29° C.
Light 80 f.c.	0.0228	0.0548	0.0574	0.0712	0.0677	0.0673	0.0751	0.0687
" 150 "	0.0272	0.0748	0.0742	0.0914	0.0976	0.1020	0.1145	0.1150
" 350 "	0.0321	0.0875	0.0810	0.1310	0.1395	0.1353	0.1618	0.1586
" 500 "	0.0337	0.0825	0.0900	0.1350	0.1520	0.1447	0.1903	0.1921
" 750 "	0.0420	0.1125	0.1084	0.1466	0.1693	0.1850	0.2134	0.2051
" 900 "	0.0460	0.1142	0.1046	0.1475	0.1816	0.1873	0.2205	0.2168
" 1100 "	0.0485	0.1108	0.1111	0.1570	0.1882	0.1747	0.2187	0.2015
" 1600 "	0.0563	0.1266	0.1158	0.1503	0.1811	0.1792	0.2254	0.2371

It must be established at the outset that the seasonal effect is negligible, so that the estimate of rate of multiplication at any combination of light and temperature is independent of the occasion on which the experiment is performed. An analysis of variance of the data at 18°, 24°, and 29° C., for which duplicates performed at different times are available, separates the effects of light and temperature on rate of multiplication from the effect of occasion. The results of the analysis are as follows:

TABLE III.

Analysis of Variance of Data at 18°, 24°, 29° C.

	Deg. freedom.	Sums of sq.	Variance.	'Z.'	1 %.
Light	7	0.068629	0.0098040	2.955	0.902
Temperature	2	0.0550745	0.0275373	3.472	2.300
Interaction light and temp.	14	0.0094043	0.0006718	1.619	0.616
Remainder(occasion)	24	0.0006372	0.0000266		
Total	47	0.1337450			

If the variance due to occasion is compared with the variances due to other factors, it is clear that the effects of light, temperature, and the interaction between them are significantly greater than any effect due to the occasion on which the experiment is performed. The duplicates at 18°, 24°, and 29°, may therefore be averaged, and the mean values given in Table IV may be taken as the basis for the discussion of rate of development.¹

TABLE IV.

Mean Regression Coefficients of Lines of Closest Fit of the Curves of log. Frond Number plotted against Time (i.e. Values for the Relative Rate of Development of Colonies of Lemna).

Temperature	10° C.	18° C.	21° C.	24° C.	29° C.	Significance ²
Light 80 f.c.	0.0228	0.0561	0.0712	0.067	0.0719	(+)
" 150 "	0.0272	0.0745	0.0914	0.0998	0.1148	(+)
" 350 "	0.0321	0.0843	0.1310	0.1374	0.1602	(+)
" 500 "	0.0337	0.0863	0.1350	0.1489	0.1912	(-)
" 750 "	0.0420	0.1110	0.1466	0.1772	0.2093	(-)
" 900 "	0.0460	0.1094	0.1457	0.1845	0.2187	(-)
" 1100 "	0.0485	0.1110	0.1570	0.1815	0.2101	(-)
" 1600 "	0.0563	0.1212	0.1503	0.1802	0.2313	(-)

The figures show that the relative rate of multiplication increases both with increasing temperature and with increasing light intensity, and the analysis shows that these increases are significant. In Figs. 6 and 7 these values are represented graphically, and a discussion of the curves will be found on page 325.

(ii) *Dry weight data.* As described in Section II the dry weights of samples of fifty fronds from each combination of light intensity and temperature were taken twice a week during each experiment. The variance among frond weights due to light and temperature must be separated from the variance due to time of sampling within the experiment, and to occasion of the experiment. For this purpose the values of frond weight covering the last three samplings (last ten days) of each of the duplicated experiments are set out in Table V, and in Table VI are given the variances due to the interacting factors.

From inspection of the values of 'Z' it will be seen that the variance due to occasion is insignificant. So also are the variances due to the interaction of occasion with light and temperature, and the triple interaction of light, temperature, and occasion. The variances due to light and temperature and to the interaction of these factors are significant. For the purposes of the discussion the mean values of frond weight for each combination of

¹ It is shown later (Tables VI and IX) that the effect of occasion is insignificant when compared with the random errors of sampling, i.e., the variability of the material in respect of frond weight and frond area.

² Significance of differences between consecutive totals.

TABLE V.

Values of Dry Weight per Frond for the last three Samplings of Experiments at 18°, 24°, and 29°.
Figures in mg.

Light intensity.	18°.									24°.									29°.									Totals.	Significance. ¹
	Jan. 1934.			March 1934.			April 1934.			June 1934.			April 1934.			June 1934.			April 1934.			June 1934.							
	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.					
80 f.c.	0.146	0.137	0.156	0.112	0.109	0.101	0.072	0.064	0.064	0.076	0.094	0.070	0.080	0.073	0.068	0.059	0.067	0.065	1.613	(+)									
150 "	0.174	0.169	0.185	0.166	0.189	0.116	0.101	0.107	0.106	0.103	0.125	0.112	0.084	0.086	0.087	0.071	0.075	0.082	2.138	(+)									
350 "	0.200	0.310	0.341	0.321	0.341	0.374	0.166	0.174	0.202	0.142	0.173	0.180	0.131	0.153	0.137	0.111	0.131	0.130	3.717	(+)									
500 "	0.287	0.410	0.590	0.406	0.440	0.430	0.195	0.205	0.223	0.187	0.185	0.200	0.156	0.185	0.176	0.144	0.124	0.145	4.688	(+)									
750 "	0.456	0.568	0.572	0.396	0.422	0.370	0.227	0.240	0.233	0.237	0.236	0.230	0.191	0.217	0.190	0.188	0.195	0.190	5.358	(+)									
900 "	0.431	0.489	0.411	0.451	0.508	0.507	0.259	0.258	0.280	0.271	0.236	0.255	0.209	0.256	0.244	0.214	0.198	0.214	5.718	(-)									
1100 "	0.431	0.545	0.370	0.473	0.479	0.461	0.288	0.273	0.270	0.266	0.209	0.252	0.219	0.261	0.239	0.200	0.187	0.230	5.653	(+)									
1600 "	0.457	0.519	0.475	0.482	0.509	0.396	0.304	0.323	0.327	0.260	0.258	0.288	0.238	0.282	0.243	0.214	0.230	0.250	6.073	(+)									
Totals	17.406									9.633									7.919										

¹ Significance of differences between consecutive totals.

light and temperature are set out in Table VII. The graphs of these values and the discussion of them are to be found on page 333.

TABLE VI.

Analysis of Variance of Frond Weights from Table V.

	Deg. free.	Sums of sq.	Variance.	'Z.'	1 % points.
Light	7	1.136108	0.162301	2.442	0.866
Temperature	2	1.065003	0.532502	3.036	2.300
Light and temp. interaction	14	0.169507	0.012108	1.144	0.550
Occasion	1	0.008740	0.008740	0.981	4.379
Light and occasion interaction	7	0.000457	0.000229	3.440	0.866
Temp. and occasion interaction	2	0.010385	0.005192	2.162	2.300
Light and temp. and occasion, interaction	14	0.041074	0.002934	0.435	0.550
Remainder (Samplings within occasion)	96	0.117852	0.001228		
Total	143	2.549126			

TABLE VII.

Mean Values for Dry Weight per Frond. Figures in milligrams. Each Entry is the Mean of six Observations from Table IV, together with the Mean Values from the non-replicated Experiments at 10° and 21°.

Temperature.	10° C.	18° C.	21° C.	24° C.	29° C.
Light 80 f.c.	0.169	0.127	0.095	0.073	0.069
" 150 "	0.264	0.167	0.126	0.109	0.081
" 350 "	0.373	0.315	0.228	0.173	0.132
" 500 "	0.531	0.427	0.245	0.199	0.155
" 750 "	0.549	0.464	0.282	0.234	0.195
" 900 "	0.575	0.466	0.315	0.264	0.223
" 1100 "	0.525	0.460	0.316	0.260	0.223
" 1600 "	0.555	0.476	0.348	0.293	0.243

(iii) *Data for frond area.* At the same time as the colonies were sampled for dry weight, corresponding samples were used for the measurement of frond area, so that at any time the total dry weight and the total frond area of the colony could be calculated. In the following tables the same type of analysis has been employed as was used for the dry weight data. From the last three samplings of the duplicated experiments at 18°, 24°, and 29° the variances due to occasion and to time of sampling within the experiment are separated from those due to light and temperature. The observations, their analysis, and the table of means, are set out in Tables VIII, IX, and X. The analysis shows (Table IX) that the variances due to occasion and its interactions are not significantly greater than that due to sampling errors within the experiment. The only significant variance in the table is that due to light. The mean values for frond area are discussed on page 332.

TABLE VIII.

Values of Frond Areas for the last three Samplings of Experiments at 18°, 24°, and 29°.
Figures in sq. mm.

Light intensity.	18°.						24°.						29°.						Totals.	Significance. ¹
	Jan. 1934.			March 1934.			April 1934.			June 1934.			April 1934.			June 1934.				
	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.	I.	II.	III.		
80 f.c.	8.7	8.5	9.1	6.4	6.8	7.9	6.1	5.9	5.6	5.8	5.4	5.3	7.5	6.1	5.9	7.7	7.3	6.1	122.1	(+)
150 "	9.6	8.6	8.9	8.0	8.2	8.0	6.4	7.0	6.7	6.5	7.8	7.2	8.1	7.2	7.3	7.2	7.1	6.9	136.7	(+)
350 "	10.6	10.2	10.1	9.7	9.5	9.1	7.5	8.1	8.3	7.9	7.8	8.4	8.1	7.9	8.9	7.9	8.3	7.3	155.6	(-)
500 "	9.0	11.4	10.8	9.4	9.0	9.9	8.1	7.5	7.6	7.3	8.1	8.0	8.0	8.0	10.2	7.6	9.2	8.2	157.3	(-)
750 "	10.4	10.3	10.6	9.4	9.0	9.4	7.9	7.1	8.3	6.8	7.8	10.3	7.1	7.9	10.0	8.2	8.0	8.7	157.2	(-)
900 "	10.3	10.7	9.2	9.9	9.4	9.8	8.1	7.0	7.2	6.6	8.0	9.8	7.8	6.9	10.1	7.9	7.9	7.9	154.5	(-)
1100 "	11.1	9.8	10.4	8.2	8.7	9.5	8.4	7.4	8.2	7.2	8.5	8.5	8.0	7.7	9.2	8.5	8.1	9.0	156.4	(-)
1600 "	9.4	9.4	9.1	8.6	8.9	8.7	7.6	7.6	7.6	8.1	7.1	9.9	6.9	6.8	10.0	7.1	7.6	8.2	148.6	(-)
Totals	447.6						361.3						379.5							
Significance ¹	(+) (-)						(+)						(-)							

¹ Significance of differences between consecutive totals.

TABLE IX.

Analysis of Variance of Frond Areas from Table VIII.

	Deg. free.	Sums of sq.	Variance.	'Z.'	1 % points.
Light	7	63·230	9·033	1·350	0·866
Temperature	2	86·226	43·113	2·131	2·300
Interaction light and temp.	14	5·329	0·381	1·267	0·550
Occasion	1	3·240	3·240	0·837	4·379
Interaction light and occasion	7	1·215	0·174	2·374	0·866
Interaction temp. and occasion	2	10·850	5·425	1·095	2·300
Light and temp. and occasion	14	4·162	0·297	1·136	0·550
Remainder (samplings within occasion)	96	58·370	0·608		
Total	143	232·622			

TABLE X.

Mean Values for Frond Area. Figures in sq. mm. Each entry is the Mean of six Observations from Table VIII, together with the Mean Values from the non-replicated Experiments at 10° and 21°.

Temperature.	10° C.	18° C.	21° C.	24° C.	29° C.
Light 80 f.c.	7·7	7·9	5·9	5·7	6·8
" 150 "	8·9	8·6	6·8	6·9	7·3
" 350 "	8·8	9·9	8·0	8·0	8·1
" 500 "	9·9	9·9	7·8	7·8	8·5
" 750 "	9·9	9·9	8·0	8·0	8·3
" 900 "	10·5	9·9	7·6	7·8	8·1
" 1100 "	10·7	9·6	8·0	8·0	8·4
" 1600 "	9·8	9·0	7·1	8·0	7·8

It will be seen from the values of 'Z' in Table IX that the variance due to occasion is insignificant, as also are the interactions in which occasion takes part. In addition, the variance due to the interaction of temperature and light is insignificant, and that due to temperature is not significant on the 1 per cent. points. In other words, frond area is apparently independent of temperature between 24° and 29° C. The only significant variance is that due to light, and inspection of the light totals in Table VIII will show that this variance lies entirely in the range of light intensities from 80 to 350 foot candles, above which area is independent of light intensity.

The statistical analysis is summarized in Table XI, in which values of 'Z' are tabulated against both 1 per cent. and 5 per cent. points.

(iv) *Assimilation rate.* From the data already given it is possible to calculate the rate of increase of dry weight of a colony and the rate of increase of area. From these the net assimilation rates can be calculated by dividing the increase in dry weight over unit time by the average area over that time. The average area, assuming growth rate to be exponential,

is merely the absolute increase in area in unit time divided by the relative rate of increase over that time (7). The values of assimilation rates calculated in this way, are given in Table XII. On the assumption that the dry weight gain is carbohydrate, the values have been expressed as milligrams of CO₂ per square decimetre per hour, to make them easily comparable with the data of other workers. It is not possible to obtain any accurate estimate of the errors attached to these values, but since they are calculated from the *mean* values of the data already analysed, there is no doubt that the striking effects of light and temperature on assimilation rate are real effects and not due to random fluctuations.

TABLE XI.

Values of 'Z', showing Significance of Light, Temperature, and Occasion upon the Growth Processes studied.

Due to	Deg. free.	Regress. coeff.	Area per frond.	Weight per frond.	1 %.	5 % (n = ∞).
Light . . .	7	2.955	1.350	2.442	0.866	0.586
Temperature . .	2	3.472	2.131	3.036	2.300	1.485
Occasion . . .	1	—	0.837	0.981	4.379	2.769
Light and temp. .	14	1.619	1.267	1.144	0.550	0.378
Light and occasion .	7	—	2.374	3.440	0.866	0.586
Temp. and occasion	2	—	1.095	2.162	2.300	1.485
Light and temp. and occasion . . .	14	—	1.136	0.435	0.550	0.378

TABLE XII.

Net Assimilation Rates, calculated from the Increase in Dry Weight per Unit Mean Area. Values given in mg. CO₂ per sq. dm. per hour.

Temperature. Light intensity.	10° C.	18° C.	21° C.	24° C.	29° C.
80 f.c.	0.710	1.269	1.611	1.216	1.027
150 "	1.154	2.035	2.380	2.218	1.792
350 "	1.905	3.811	5.250	4.180	3.662
500 "	2.560	5.230	5.967	5.363	4.905
750 "	3.259	7.319	7.237	7.559	6.281
900 "	3.538	7.246	8.489	8.785	8.471
1100 "	3.378	7.482	8.718	8.299	7.847
1600 "	4.452	9.017	10.360	9.285	10.137

In Figs. 8 and 9 these values are represented graphically, and a discussion on them will be found on page 328.

Experiments in 1933. These were preliminary to the series already described. Owing to difficulties in maintaining a constant environment, and in sampling from sixteen colonies simultaneously, the data are incomplete. The data obtained confirm, however, those set out in this paper. In addition, one experiment was performed at 35° C. Frond numbers were taken from the first day of the experiment, and frond areas and frond weights on three occasions during the experiment. Both relative

multiplication rate and frond area fell with time. In Fig. 5 the logarithms of frond number are plotted against time for four of the light intensities.

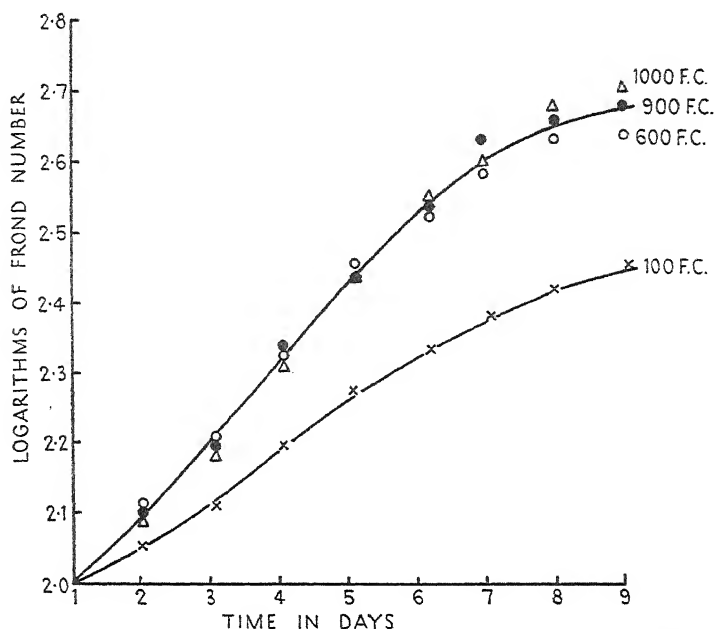


FIG. 5. Experiment at 35°C. logarithms of frond number plotted against time in days.

It seems that at this temperature a factor deleterious to growth operates by retarding the rate at which new primordia are formed, and reducing the frond area.

IV. DISCUSSION OF DATA.

The data analysed in the previous section are organized under four heads: relative multiplication rate, assimilation rate, frond weights, and frond areas. In this section the relevant data are represented graphically, together with a formal discussion of their significance. A more exhaustive treatment of the data is deferred until assimilation and respiration studies at present in hand in this laboratory are completed.

(i) *Relative multiplication rate.* The logarithms of frond number plotted against time fall on straight lines, the slopes of which are taken as measures of the relative rate of frond multiplication in the different colonies (p. 317). The values for these slopes are plotted against temperature in Fig. 6, and light intensity in Fig. 7. In Fig. 11 the two graphs are seen combined in a model, the surface of which records the relative multiplication rate under the forty different combinations of light and temperature at which growth was exponential.

It will be seen from Fig. 6 that the relation between relative multiplication rate and temperature is approximately linear, except at 80 and

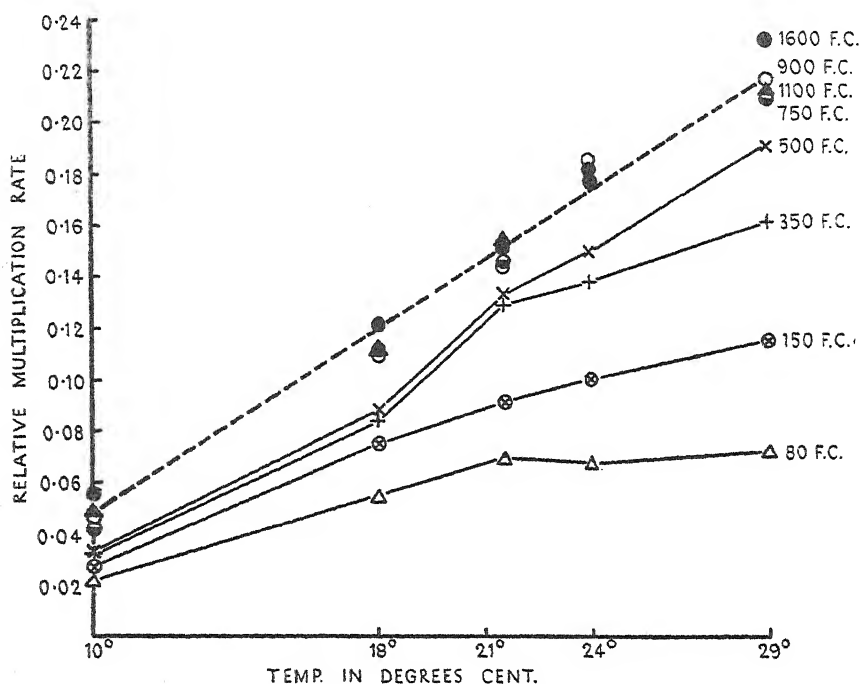


FIG. 6. Relative multiplication rates plotted against temperature, from values in Table IV.

150 foot candles. This indicates that the temperature coefficient decreases continuously with rise of temperature. The values of Q_{10} for various intervals are given in Table XIII.

TABLE XIII.

Temperature Coefficients of Relative Multiplication Rate.

Light.	10°–18° C.	18°–24° C.	24°–29° C.
80	3.08	1.36	1.14
150	3.53	1.63	1.32
350	3.35	2.27	1.36
500	3.25	2.49	1.64
750	3.57	2.18	1.39
900	2.96	2.39	1.40
1100	2.82	2.27	1.34
1600	2.61	1.94	1.65

There is no obvious explanation of the falling temperature coefficient with rising temperature. The values for the lower temperatures are consistent with the hypothesis that the rate of formation of fronds depends on

some chemical process. At higher temperatures the lower values for Q_{10} may be attributed to the limiting of the rate of development by (i) a photo-

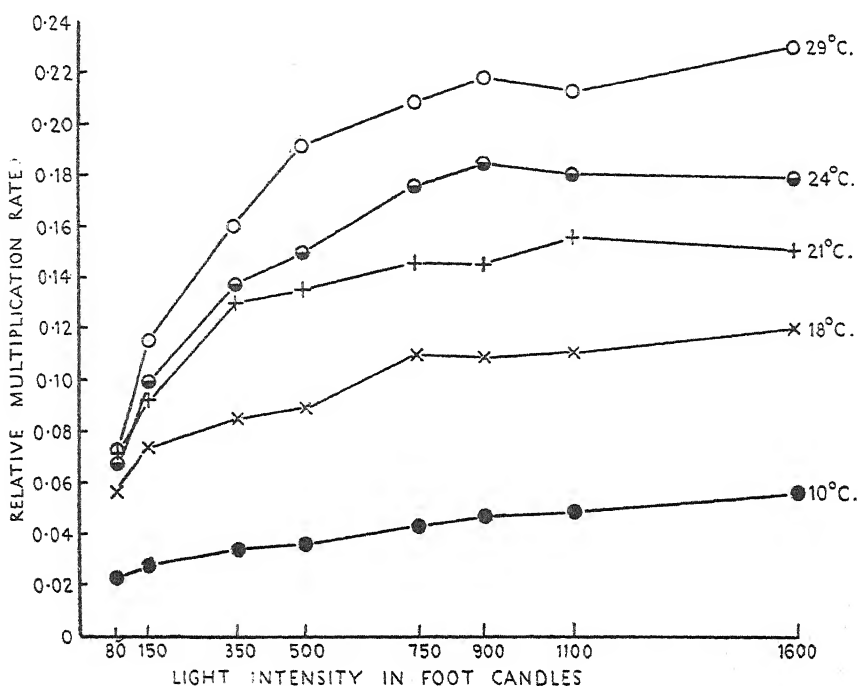


FIG. 7. Relative multiplication rates plotted against light intensity, from values in Table IV.

chemical process, or (ii) a physical process. That a photochemical process alone should be controlling seems unlikely, since the values of the temperature coefficient do not rise with increasing light intensity, and the high value at 80 foot candles, from 10° to 18°, indicates that even here light is not controlling rate of multiplication. On the other hand, physical processes, such as diffusion of salts to the absorbing surface of the plant or the viscosity of protoplasm within the plant, may control the rate of multiplication at higher temperatures. It seems that the rate at which nutrient salts are supplied is adequate, since diluting the culture solution does not reduce the relative multiplication rate. Fauré-Fremiet (5) suggests that the viscosity of protoplasm may limit the rate of cell-division at higher temperatures, and this may be put forward as a tentative and possible suggestion to account for the falling temperature coefficient.

In view of the subsequent discussion it should be noticed that the multiplication rate is still rising significantly from 24° to 29°.

In Fig. 7 the relative multiplication rates are plotted against light intensity. Reference to the analysis on p. 319 will show that the rise of

multiplication rate with light intensity is significant up to 750 foot candles, but that above this light intensity there is no further increase in growth rate. The curves are approximately exponential in type. Above 750 foot candles temperature controls the multiplication rate, but below this the rate is

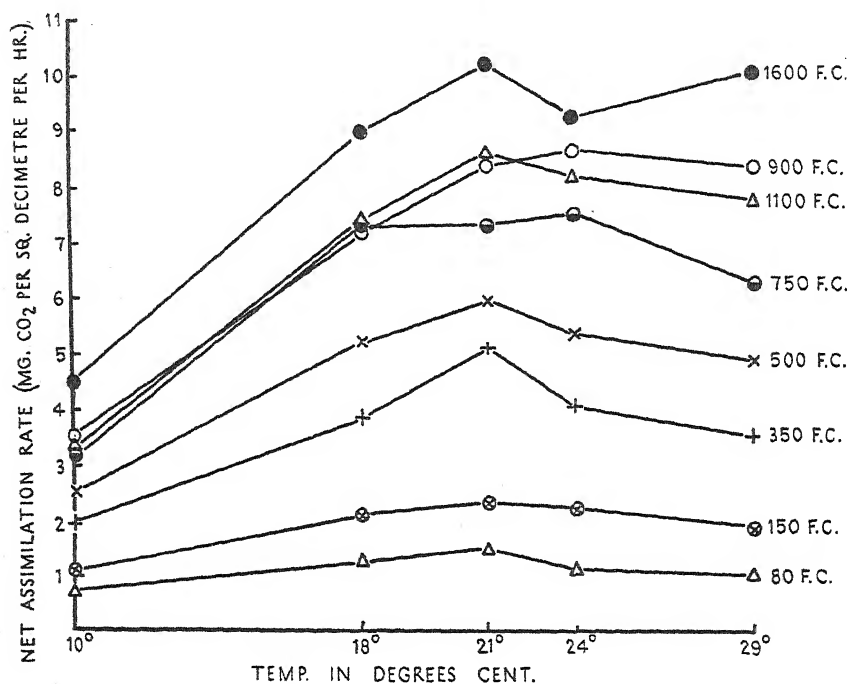


FIG. 8. Mean net assimilation rates in milligrams of CO_2 per square decimetre per hour plotted against temperature.

clearly affected by light intensity. This effect is not due to the limiting of the growth by shortage of assimilates, for even at 150 foot candles both free reducing material (probably monosaccharide) and abundant starch are present. Further evidence for the independence of multiplication rate and assimilate will be advanced immediately. It must be concluded, therefore, that light intensity controls the multiplication rate through some process other than assimilation.

(ii) *Assimilation rate.* The net assimilation values in milligrams of CO_2 per square decimetre per hour for colonies grown under the various conditions are given in Table XII; in Figs. 8 and 9 these are represented graphically.

It is evident from Fig. 8 that assimilation rates rise from 10° to 18° at all light intensities, but that between 18° and 29° the assimilation rate is independent of temperature. This may be explained on the supposition

that the photochemical reaction is controlling the rate of assimilation above 18°, so that an increase in temperature will not increase the rate of the 'dark' reaction, because light is limiting the supply of the necessary reactant. Even at 10° C. there is a marked effect of light on assimilation, which is therefore not completely determined by the 'dark' reaction at this temperature. This is in accordance with expectation if the 'dark' reaction follows a photochemical reaction, since increase in light will increase the concentration of the reactants consumed in the 'dark' reaction, and hence increase the velocity of assimilation whatever the temperature.

TABLE XIV.

Temperature Coefficients of Net Assimilation Rate.

Temperature.		10°-18° C.	18°-24° C.	24°-29° C.
Light	80	2.07	0.99	0.97
"	150	2.04	1.17	0.98
"	350	2.38	1.17	0.99
"	500	2.45	1.04	0.99
"	750	2.75	1.05	0.98
"	900	2.46	1.37	1.00
"	1100	2.70	1.19	0.99
"	1600	2.42	1.05	1.19

It is of interest to compare the curves in Fig. 8 with those in Fig. 6. Whereas the assimilation rate is independent of temperature from 18° to 29° the relative multiplication rate is nearly doubled over the same range. It is clear that the relative multiplication rate is not dependent upon the assimilation rate, a point similar to that brought out by Gregory in his work on leaf growth of barley (7). The temperature coefficients of assimilation rate are tabulated in Table XIV. It is to be observed that the Q_{10} is independent of light intensity, and a comparison of these and the corresponding values for multiplication rate (Table XIII) gives further indication that the one process is not rigidly controlled by the other.

The assimilation values are plotted against light intensity in Fig. 9. The values, except those at 10° C., approximate to one curve which is still rising at 1,600 foot candles. The maximum light intensity for assimilation has not been reached. Comparison with Fig. 7 shows that whereas the assimilation rate is still rising at 1,600 foot candles the relative multiplication rate does not rise significantly above 750 foot candles: another illustration of the relative independence of assimilation and multiplication rates.

The above features are summarized in Figs. 10 and 11, where the light and temperature curves are combined into three-dimensional models.

The direct relation between assimilation rate and growth rate may be seen from Fig. 12, where the relative multiplication rates are plotted against the corresponding assimilation rates. It is clear from this figure that a wide

range of multiplication rates may occur at any one assimilation rate. For example, at an assimilation rate of 3 mg. CO₂ per square decimetre per hour the multiplication rate may vary from 0.042 at 10° C. to 0.149 at 29° C. It is possible to read off from the figure the limits within which multiplication rate is independent of assimilation level.

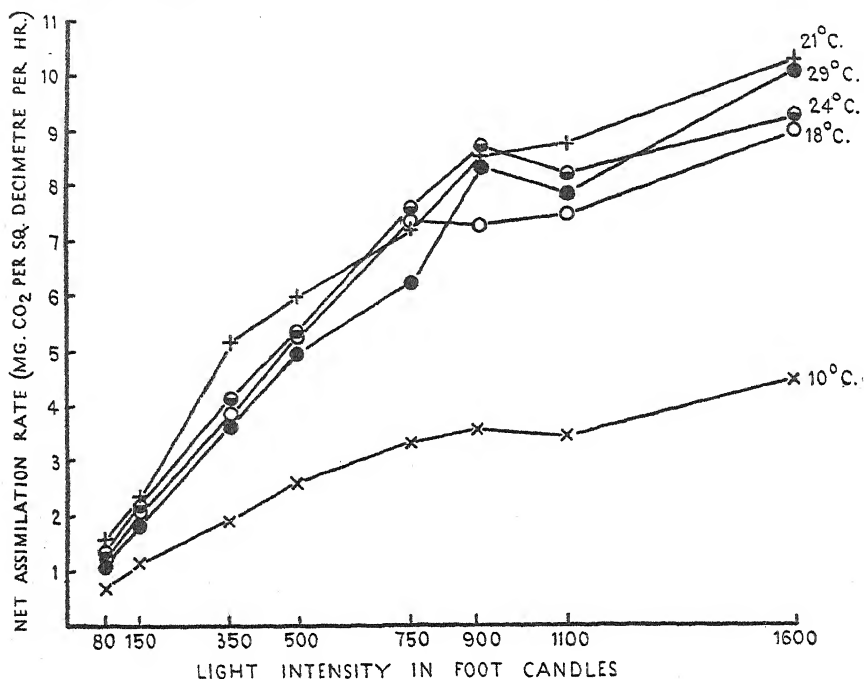


FIG. 9. Mean net assimilation rates in milligrams of CO₂ per square decimetre per hour plotted against light intensity.

At temperatures slightly higher than 29° C. a factor deleterious to growth operates. Hence the curve at 29° C. represents at all levels of assimilation the maximum relative growth rate obtainable for the conditions of the experiment, since a change of temperature in either direction reduces the multiplication rate.

The curves approximate to logarithmic decrement curves ('Mitscherlich curves') of the form :

$$r_t = R_t(1 - e^{-ks})$$

where R is the maximum multiplication rate at a temperature t , and r is the multiplication rate at any assimilation level s , whereas k is a constant. This equation may be written in the form :

$$\log \frac{R-r}{R} = -ks$$

i.e. the logarithms of decrements of each point from the maximum of its particular curve, plotted against the corresponding assimilation value, should

give a series of parallel straight lines of slope k , each line cutting the ordinate at the value of $\log R$ for its particular temperature. That this is approximately true for temperatures from 18° to 29° is evident from Fig. 13. The

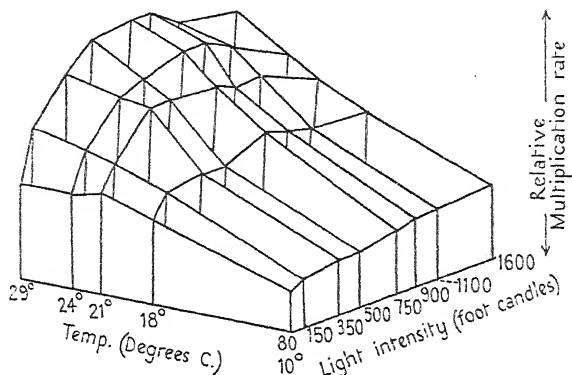


FIG. 10. Drawing of a three-dimensional model representing the interaction of light and temperature on relative multiplication rate.

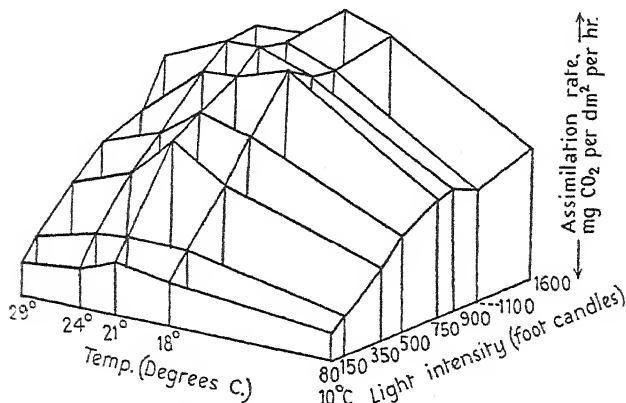


FIG. 11. Drawing of a three-dimensional model representing the interaction of light and temperature on mean net assimilation rate.

multiplication rate may therefore be expressed in terms of the assimilation level, the temperature, and the maximum multiplication rate at that temperature.

Since the curves in Fig. 12 are all logarithmic decrement curves with the same exponential constant, the temperature coefficient of multiplication rate at any given assimilation rate will be independent of the assimilation level chosen, i.e.

$$\frac{r_{t_1}}{r_{t_2}} = \frac{R_{t_1}(1 - e^{-ks})}{R_{t_2}(1 - e^{-ks})} = \frac{R_{t_1}}{R_{t_2}}$$

If c is the temperature coefficient the relation between R_t and R_{max} is:

$$R_t = R_{max} \cdot c^{\frac{t - t_{max}}{10}}$$

It is possible therefore to express the multiplication rate at any temperature for any assimilation level in terms of the maximum value at 29° C.

(iii) *Dry weight per frond*. The analyses on p. 320 show that light intensity and temperature have a significant effect on frond weight, and

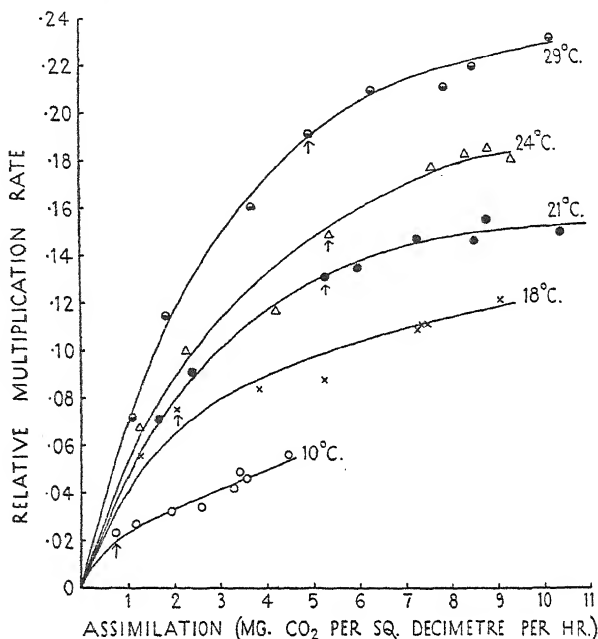


FIG. 12. Relative multiplication rate plotted against assimilation rate at different temperatures. The arrows indicate the assimilation levels at which starch appears in the fronds. The curves are drawn through the points by eye.

also that the interaction of light and temperature is significant. Since the size of a frond varies very little under the experimental conditions, the dry weight per frond may be taken as a rough measure of the amount of stored starch.

In Fig. 14 the values for mean frond weight are plotted against light intensity. The frond weights rise significantly up to 1,600 foot candles. This is in accordance with the differential effect of light on assimilation and multiplication rate. The rise is less marked at 10° C., where the assimilation rate is relatively insensitive to change of light intensity (Fig. 9).

In Fig. 15 the frond weights are plotted against temperature. The highest frond weight is at the lowest temperature, and the weights fall steadily to 29° C. The maximum storage of starch thus takes place at the lowest temperature. The frond weight represents merely the balance between assimilate formed and consumed in multiplication of fronds.

(iv) *Area per frond*. The analysis on p. 322 shows that the frond area is independent of temperature from 24° to 29° C., and independent of light

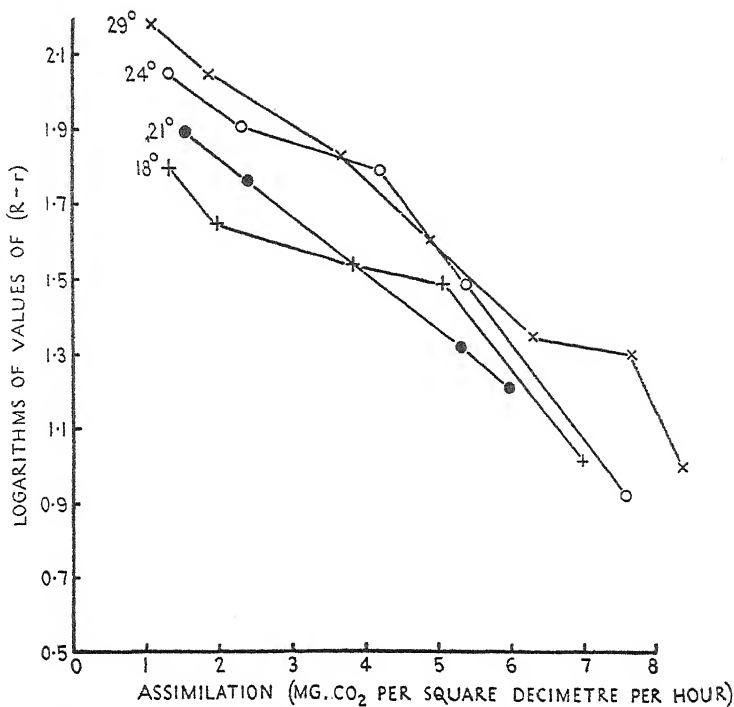


FIG. 13. Logarithms of values of $(R-r)$ for different temperatures plotted against corresponding assimilation rates. For explanation of symbols see text, p. 321.

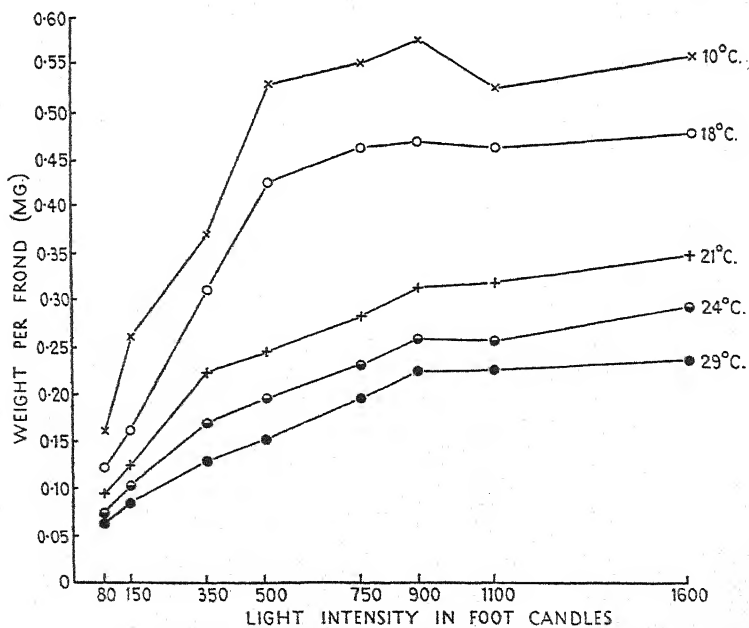


FIG. 14. Mean dry weight per frond plotted against light intensity.

intensity above 350 foot candles. Microscopic investigation of the epidermal cell size of fronds grown at 15°, 20°, and 30° C., shows no difference in the cell size under these three conditions, and shows further that cell-division falls off exponentially with the expansion of the frond in such a way that

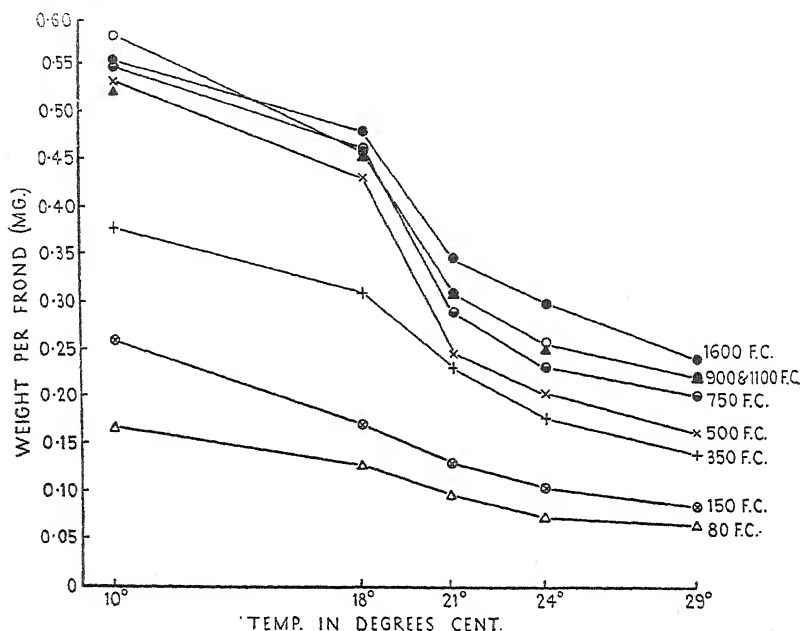


FIG. 15. Mean dry weight per frond plotted against temperature.

very few cell-divisions take place after the frond has appeared. Hence the frond area is determined by the number of cells in the primordium, and this is independent of temperature and light intensity within the limits stated above. This is in agreement with data secured by Ashby in 1928 and Hicks in 1930 (10).

The *total* area of a colony, on the other hand, is determined by the temperature and light intensity, for since the frond area remains constant, the total area of a colony is proportional to the number of fronds present. Gregory points out in his work on cucumbers (8) that the temperature determines leaf area only in so far as it affects the rate of development of the primordia. The same conclusion may be drawn from the present data. It appears that temperature acts on area simply through its effect on the frequency with which new primordia appear in the colony.

V. SUMMARY.

An apparatus is described in which colonies of *L. minor* can be grown under sixteen combinations of light intensity and temperature. A descrip-

tion is given also of a new apparatus for the rapid measurement of frond area.

A technique is detailed for the investigation of frond area, frond weight, and the rate of multiplication of fronds in the experimental colonies.

Data are given for the relative multiplication rate of fronds (relative growth rate), for frond weight, and for frond area, of colonies grown under forty combinations of conditions: at five temperatures from 10° to 29° , and at eight light intensities from 80 to 1,600 foot candles. Duplicate experiments were carried out under twenty-four of these conditions to test for any seasonal drift in the behaviour of the colonies. Data are given also for an experiment at 35° .

The data are subjected to statistical analysis with the following results:

(i) The 'occasion' on which the experiment is performed has no significant effect upon the growth processes studied, so long as the colonies are kept under controlled conditions between each experiment.

(ii) Light intensity and temperature have a significant effect upon relative multiplication rate and upon frond weight. The variance due to the interaction of light and temperature on these processes is also significant.

(iii) Frond area is independent of temperature above 24° and of light intensity above 350 foot candles.

From the data the following conclusions are drawn:

(i) The net assimilation rate, calculated on a basis of the increment in dry weight, increases linearly with light intensity over the whole range examined, but is independent of temperature between 18° and 29° C.

(ii) The relative multiplication rate of fronds increases linearly with temperature up to 29° C., but does not increase significantly with light above an intensity of 750 foot candles. Light and temperature, therefore, affect assimilation rate and growth rate quite differently. It is thus clear that the relative multiplication rate is not completely controlled by the assimilation rate over the range of conditions studied. The relation between relative multiplication rate and assimilation rate is best seen from the figure in which these two quantities are plotted against one another (Fig. 12). The values of relative multiplication rate plotted against the corresponding assimilation rates follow as a first approximation a set of 'Mitscherlich curves', one for each temperature, so that the multiplication rate can always be expressed as a function of the temperature, assimilation level, and the *maximum possible* multiplication rate. Since the curves in this figure have the same exponential constant, the temperature coefficient of multiplication rate at any assimilation rate is independent of the level of assimilation chosen.

(iii) At light intensities as low as 150 foot candles both starch and monosaccharide may be present. Therefore even at low light intensities

increase in multiplication rate with increasing light can hardly be due solely to increase in available assimilate. It is concluded that light intensity has some direct quantitative effect upon multiplication rate other than that acting through assimilation rate.

(iv) The final area of a frond depends on the number of cells laid down in the primordium, and over a wide range this is independent of temperature and light intensity. The total area of a colony depends on temperature and light only in so far as these factors determine the rate at which new primordia are formed.

(v) At 35° a factor deleterious to growth operates. Both relative multiplication rate and frond area fall off with time, while the frond weight remains high.

It is with pleasure that the authors offer their thanks to Professor V. H. Blackman and Dr. F. G. Gregory for suggestions throughout the course of this work, and to Mr. W. Shaw for the construction of the apparatus described on p. 311.

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The 'Needles' of *Asparagus*, with Special Reference to *A. Sprengeri* Reg.¹

BY

AGNES ARBER.

With three Figures in the Text.

I. INTRODUCTION.

FROM a study of the genera *Myrsiphyllum* and *Asparagus*, published in this journal in 1924 (2), I concluded that each 'phylloclade' of *Myrsiphyllum* was the prophyll of a reduced shoot, whose axis had, as a rule, no free existence. The 'needles' of *Asparagus*, on the other hand, seemed to me to be essentially axial. In a later memoir on these genera, Stefanoff (5) accepts my view of *Myrsiphyllum*; but he interprets the needle of *Asparagus* somewhat differently, bringing it into line with the phylloclade of *Myrsiphyllum* by treating it, also, as a shoot reduced to a prophyll. The striking divergence in form between the assimilating organs of the two genera, he accounts for on the theory that, in the phylloclade of *Myrsiphyllum*, the prophyllar leaf is reduced to the lamina alone, while, in the needle of *Asparagus*, it takes the form of a petiolar phyllode. The needles of *Asparagus* are, in certain species, associated with minute, non-vascular, basal scale-leaves. I regret that, when I recorded their existence in 1924, I was unaware that Buscalioni (4) had noticed them ten years earlier. I considered that their presence provided evidence for the axial nature of the needles, but Stefanoff holds that I was wrong about the position of insertion of these vestigial structures, and hence about their interpretation. His criticism has led me to make a more thorough examination of a species possessing these scale-leaves; I have chosen *A. Sprengeri* Reg., because good material of this member of the genus, grown under glass, was available at the Cambridge Botanic Garden, through the kindness of the Director.

I will first describe my observations, and then discuss their bearing.

¹ This paper represents part of the work carried out with the aid of a grant from the Dixon Fund of the University of London.

II. OBSERVATIONS.

There is considerable variety in the general shoot structure of *A. Sprengeri*, but the examples drawn in Fig. 1 are fairly typical. Fig. 1, A shows a mature vegetative shoot, produced laterally in the axil of a membranous scale-leaf with a thorny base. The attitude of the branch is characteristic; it slopes slightly downwards, thus bending back the membranous part of the axillant leaf. At the base it bears two needles—one to right and one to left—and these are succeeded at a higher level on the axis by clusters (in this shoot, trios) of needles, each group arising in the axil of a scale-leaf. I am using the term 'needle' because it carries no morphological implication, but it happens that in this species the organs in question are much less needle-like than in most of the members of the genus. Fig. 1, B 1, shows a shoot which is of the same general type as that drawn in Fig. 1, A, except that it is fertile. Two infructescences arise basally, one on either side, in association with the two basal needles. In shoots like these, studied at maturity, certain vestigial leaves can still be seen in a chaffy condition, close to the insertion of the needles, but their relations cannot be determined at this stage; for a complete analysis, sections of the young bud in spring are also needed. In Fig. 2, sections are drawn from a series passing upwards from below through a rudimentary lateral branch from a shoot collected in May; this lateral would have developed into a fertile branch of the type sketched in Fig. 1, B 1. In Fig. 2, 1, the axillant leaf is shown, just separating from the axis of the first order. It remains attached to the axis by its margins and midrib at a level at which the regions between have become free, so that two basal pockets are formed. It is in these basal pockets that the first two lateral buds of the third order are produced. There is little doubt that their position to right and left is simply an effect of the peculiar space conditions. In this series of sections, these buds have no obvious axillant leaves, but in Fig. 3, A 1 and A 2, a basal bud of this type is seen completely enclosed by the margin of the non-vascular prophyll of the lateral shoot of the second order. It thus seems reasonable to treat these basal shoots as two collateral buds axillary to the prophyll. In Figs. 2, 2 and 3, each of these shoots itself bears a non-vascular prophyll, which contains in its axil a rudimentary inflorescence bearing two leaves; this is most clearly visible in Fig. 3, B. The basal shoot also bears a second non-vascular leaf, which I consider to be axillary to the basal needle; this identification will be considered further in the next Section. I regard the inflorescence and needle on either side of the shoot base (shown externally in Fig. 1, B 1, and in section in Fig. 2, 2-5) as shoots of the fourth order, arising laterally on an extremely reduced axis of the third order, which divides itself wholly between them. Variations on this arrangement are found; for example, a basal shoot may

be developed on one side only (Fig. 3, A 1-A 6), or the basal shoot may produce an inflorescence but no needle (Fig. 3, B). Moreover, fusions may occur between leaves belonging to shoots of different orders; the prophyll of the basal shoot may fuse with the prophyll of the inflorescence,

ASPARAGUS SPRENGERI Reg (Shoots in October)

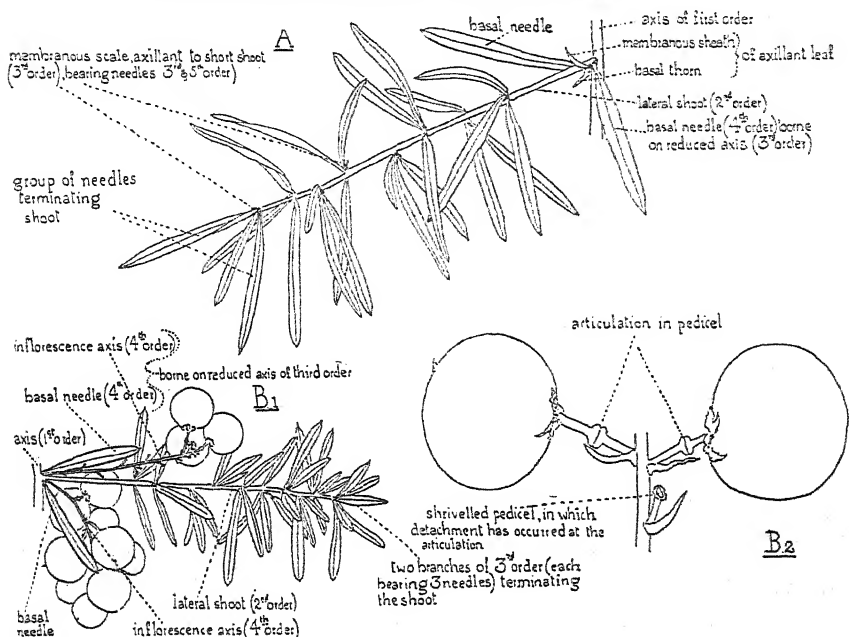


FIG. 1. *Asparagus Sprengeri* Reg., from plants grown under glass, Cambridge Botanic Garden, October, 1934. The term 'axis of the first order' is used throughout the figures illustrating this paper for the axis of the earliest order which is included, though its order in the total branch system of the plant is unknown. A, vegetative shoot (about natural size). B₁, fertile shoot (about natural size). B₂, part of an infructescence axis with two berries (magnified) to show the articulation of the pedicel; below the berries is a shriveled pedicel in which detachment has occurred at the plane of the articulation.

or with the axillant leaf of the basal needle (Fig. 3, A 4); or the axillant leaf of the basal needle may fuse with the prophyll of the lateral shoot of the second order.

Above the basal shoots, the lateral branch bears a series of scale-leaves with short shoots of the third order in their axils (Fig. 2, 6-8). As far as can be judged from such young material, the first nine leaves have shoots of the five-needled type in their axils, while the succeeding seven leaves bear needle trios, and the seventeenth and last shoot is two-needled. The first two axillary leaves occur approximately in the lateral plane, while the third and fourth lie more or less in the antero-posterior plane. From the fifth leaf onwards, the arrangement tends more to spiralness. Fig. 2, 8,

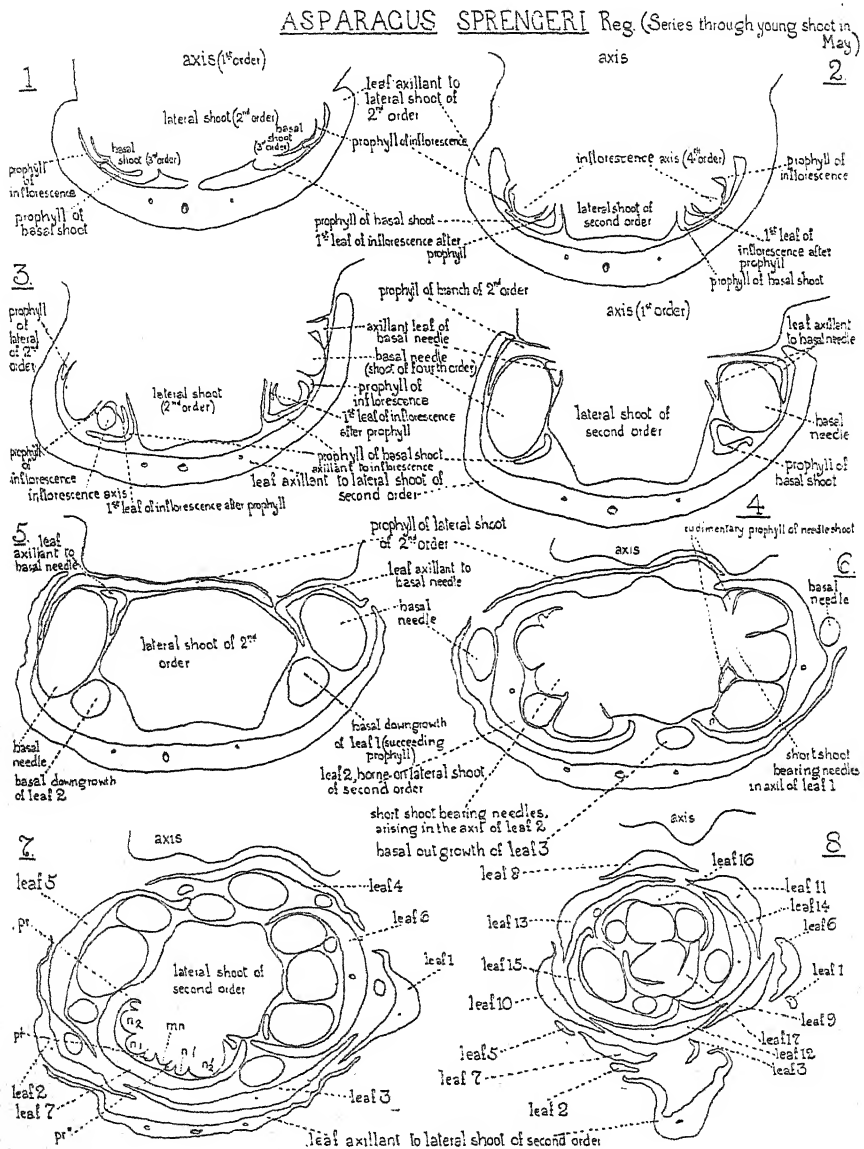


FIG. 2. *Asparagus Sprengeri* Reg. 1-8, transverse sections from a series from below upwards through a young lateral shoot, collected on May 4, 1934, from a plant grown under glass, Cambridge Botanic Garden ($\times 47$). The vascular system is not indicated except in the leaf members. In 1, the axillant leaf is not yet completely free from the axis; 2-5 show the origin of the rudimentary inflorescences and the basal needles; 6 (from more than one section)-8, show the further history of the shoot; 8 passes through the apical region. No sections are included between the base of the seventh and seventeenth leaves. For explanation of lettering in 7, see p. 343.

represents a section in the neighbourhood of the shoot apex, and shows all the leaves after the prophyll (except the fourth, which does not happen to reach to this level). The whole of the shoot apex is divided between the sixteenth and seventeenth leaves, and their axillary branches; no residual axial tissue is left between. The shoot apex, if it had been allowed to arrive at maturity, would thus have corresponded almost completely to Fig. 1, B 1, in which two needle trios, in the axils of two scale-leaves, form the termination of the shoot.

III. DISCUSSION.

Stefanoff's criticism of my view of the needles of *Asparagus* is closely bound up with the interpretation of the vestigial leaves associated with them in *A. Sprengeri* and certain other species. The extreme reduction, both of the vestigial leaves, and of the axes of higher orders, makes the situation so puzzling that no explanation of the shoot system can pretend to be anything but provisional. The complication is, moreover, increased by the fusion between vestigial leaves belonging to axes of different orders, to which I have drawn attention above, and to which Buscalioni, also, has referred. It is probable that the young shoot develops under considerable pressure. I have seen the margins of the first and second leaves actually moulded into a concave form upon the downgrowth of leaf 3 which comes between them. Such pressure may account for the unions which occur between the delicate, non-vascular scales. In the diagrams I have concentrated upon the basal region of a lateral shoot of the second order, because the problem of the nature of the vestigial leaves, and their relation to the needles, is here presented in a simplified form, since there are generally two basal needles only.

To make the descriptions in the preceding Section clearer, I have anticipated this Discussion so far as to give provisional identifications of the various vestigial leaves in the basal region of the shoot drawn in Figs. 2, 1-5, and 3, A and B. Two points of theoretical interest arise out of these identifications. The first of these points concerns the rudimentary scales which, from a comparison of my sections with the mature structures, I now regard as leaves borne on the inflorescence axis; they are shown in Figs. 2, 2 and 3, and the left-hand basal shoot in Fig. 3, B. In the light of these sections, I have now come to the conclusion that certain members in *A. Sprengeri*, which I formerly described as needles bearing vestigial leaves (2, w and x, in Figs. 30 and 31, p. 651), were more probably inflorescence axes in their most rudimentary stages; my statement in that paper that the needles sometimes bear as many as three small-scale leaves must thus be cancelled. The second point relates to the two basal needles. These, which are borne upon the basal shoots of the third order, are each associated

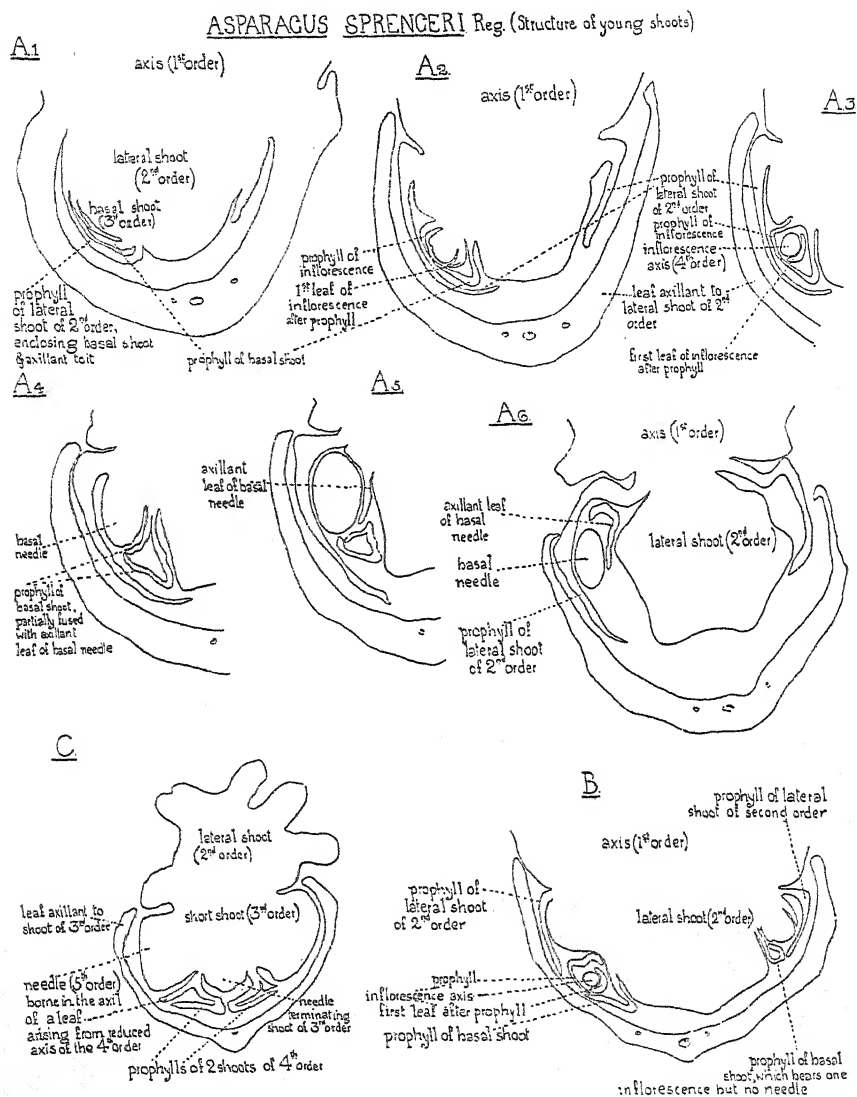


FIG. 3. *Asparagus Sprengeri* Reg. A and B, sections ($\times 47$) from transverse series from below upwards through other buds from the same lateral shoot as that shown in Fig. 2. A 1-A 6, a shoot with only one basal lateral shoot of the third order, which is enclosed in the margin of the prophyll of the shoot of the second order. B, a lateral bud in which the right-hand basal shoot bore an inflorescence, but no needle. C, transverse section of a shoot passing through the base of a needle trio, somewhat older than those shown in Fig. 2, 6 and 7 ($\times 47$). Throughout these diagrams, the vascular system is indicated in the leaf members only.

with a vestigial leaf (Fig. 2, 4 and 5; Fig. 3, A 4-A 6). Buscalioni (4), who takes the foliar view of the *Asparagus* needle, has suggested that, where such a vestigial leaf is associated with the needle, the needle and scale

together are equivalent to the ordinary *Asparagus* leaf borne on one of the long axes—the needle corresponding to the spine, and the scale to the ligular sheath. But I do not think that either the position of the vestigial leaf, or its relation to the needle, in the sections which I have described, are such as to justify the view that it is a ligular outgrowth belonging to the needle. It seems to me more reasonable to suppose that this vestigial leaf is the second leaf arising from the axis of the third order, and bearing the basal needle in its axil, just as the first leaf (the prophyll) bears the inflorescence. On this view, the whole apex of the basal shoot of the third order is employed in producing these two leaves and their axillary buds, exactly as, at the apex of the branch of the second order, two needle groups with their axillant leaves terminate the shoot, leaving no trace of a growing point between (Fig. 2, 8).

In the purely vegetative needle groups, occurring above the basal region which we have been considering, five needles may be taken as the full and typical development (cf. the group of mature needles sketched in 2, Fig. 29, p. 651). Such a short shoot, in its embryonic stage, is seen in section in Fig. 2, 7; leaf 7 is axillant to it, and it bears a prophyll, *pr.* The relation of the parts in the needle cluster can be understood if we regard the scheme as a version of that of the basal region. The median needle, *m.n.*, may be held to replace the axis of unlimited growth of the lateral shoot of the second order in Fig. 2, 3, &c. The needles *n.₁* and *n.₁'* arise in the axils of *pr.*' and *pr.*", the prophylls of the shoots of the fourth order, thus replacing the inflorescence axes, while *n.₂* and *n.₂'* correspond to the basal needles. The three-needled groups are reduced from the five-needled type by the absence of the needles corresponding to the inflorescence axes. Fig. 3, C, is drawn from a needle trio which is older than the needle groups shown in Fig. 2; the details are thus more clearly distinguishable. It will be seen that the prophyll of the short shoot on either side is fused with the axillant leaf of the lateral needle; a fusion of the corresponding vestigial non-vascular leaves in the basal region is seen in Fig. 3, A 4.

If my identifications of the various vestigial leaves, and of the associated axes, be accepted, they confirm the theory of the shoot nature of the needle, but we have still to consider whether the shoot in question is reduced to an axis alone, or, as Stefanoff suggests, to a petiolar phyllode. The latter alternative has the attraction of bringing *Asparagus* into line, not only with *Myrsiphyllum*, but also with *Danae*, *Ruscus*, and *Semele* (1); but it is difficult to prove. Stefanoff points to the articulation of the needle as a distinctively foliar character, but much weight cannot be attributed to this argument, since the articulation can be paralleled in the pedicel of *Asparagus* itself, though here it is not basal (Fig. 1, B 2). He also regards the anatomy of the needle as characteristically petiolar; but though the structure does not preclude the petiolar view, I do not think that it shows

any features of which examples cannot be found among axes. I doubt, indeed, if the available evidence is adequate for a final decision between the (*axis + petiole*) and the (*axis alone*) theories, but this is perhaps of no great importance, since it is the *shoot* that should be treated as the fundamental unit (3). There is something artificial in the attempt to apply the conventional descriptive terms, stem and leaf, to such an organ as the needle of *Asparagus*; it is probably better to define it simply as a reduced shoot.

IV. SUMMARY.

The shoot structure of *A. Sprengeri* Reg., which was touched upon in an earlier paper (2), has been studied afresh in greater detail in connexion with certain criticisms by Stefanoff (5). This re-examination has shown that the observations which I formerly recorded were, in part, wrongly interpreted. Though the present study thus involves a correction of some of my previous identifications, it confirms my former view that the needles of *Asparagus* are reduced shoots. The vestigial leaves, which are sometimes associated with the needles, I now regard as axillant to them.

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The Effect of Some Cations on the Permeability of Cells to Water.

BY

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With thirteen Figures in the Text.

INTRODUCTION.

A GREAT deal of physiological investigation has been concerned with the permeability of cells to various ions, but the problem of the permeability to water has not hitherto received adequate attention. Work previous to 1924 is reviewed by Stiles (9) in his book, and the chapter on permeability to water deals almost exclusively with his own work on this aspect of the problem. Since this time contributions have been made by Huber and Höfler (1), de Haan (2), and McCutcheon and Lucke (3, 4, 5). The first-named investigators employed the plasmometric method, and the permeability to water was determined by rate of change in volume of cells during plasmolysis and subsequent recovery in water. This method seems to be tedious in application and limited to such plant tissues as show regular shrinkage during plasmolysis. The third workers mentioned determined the rate of water uptake from changes in dimensions of egg cells, and subsequent calculations.

In the present work the aim has been to establish the change in the permeability to water as the result of previous exposure of cells to the effect of hypotonic solutions of nutritive ions.

Permeability may be defined as the mass of a substance passing unit area per unit time under unit concentration gradient. In the case of permeability to water the concentration cannot be defined precisely. Permeability to water will therefore be defined as rate of movement of water per unit area, per unit gradient of suction pressure. In the present work the water deficit was brought to the same level in all the experiments so that the suction pressure should be approximately the same throughout, and this was shown experimentally to be the case by the use of cane-sugar solutions.

Permeability can therefore be directly compared in these experiments by the rate of uptake of water and, since both area and initial suction pressure are the same, permeability is proportional to the rate. The rate may be looked upon as determined by a resistance to flow of water, and this resistance is inversely proportional to the permeability.

Experimental methods. Cylinders of potato tuber and carrot were cut symmetrically by means of a cork borer 1.8 cm. in diameter. These were cut by means of a hand microtome into discs of a uniform thickness of 0.5 mm., weighing approximately 150 mg. The discs of tissue were left to soak overnight in hypotonic solutions of chloride (of 1 atm. osmotic pressure) of those cations whose effect on permeability was to be studied. Previous to the soaking the discs were washed in the same solutions in order to remove the starch grains from the cut cells. They were then superficially dried between filter paper, using a weighted photographic roller to ensure uniform drying, weighed, dried over calcium chloride in closed vessels for a specified time, and the uptake in distilled water then measured. The control discs were treated in a similar way, except that they were washed in distilled water after cutting, and left to soak overnight in distilled water. The weighings were performed on a torsion balance sensitive to 1 mg. Each disc was treated individually, and in general ten replicates were used for each treatment. In this way a whole series of uptake curves were obtained. The salts used were the purest obtainable and were chlorides of the cations, Na', K', NH₄', Ca'', and Mg''. Before the initial drying over calcium chloride, the discs which had been in salt solutions were removed, dried between filter paper, and then weighed immediately. This weight was the basis from which the water deficit was calculated.

The discs were allowed to take up water for four consecutive periods of 15 seconds, and were superficially dried and weighed after each immersion. They were then immersed for two consecutive periods of 30 seconds, dried and weighed as before, and then for further periods of 60 seconds until they were found to have approached their final equilibrium as was shown by weighing after a further considerable period of time. The drying and weighing took approximately 30 seconds from the time the discs were removed from the water until they were again immersed. The actual weighing occupied less than half that time. The loss in weight of discs left exposed in air, attached to the torsion balance, and weighed at intervals of 30 seconds for a period of ten minutes, was found to be less than 0.4 mg. in 15 seconds, which is smaller than the accuracy with which the scale can be read. The timing of the immersions was by a stopwatch reading to one-fifth of a second, as these small time intervals were necessary owing to the rapid entry of water after drying.

The distilled water (glass distilled) was contained in a beaker and

kept at constant temperature at 17°C. by means of a water bath. To make the experiments as comparable as possible large potatoes were selected, so that the same tuber could be used on consecutive days. It will be seen later that agreement between results obtained under those conditions is excellent.

Thickness of disc. The aim throughout these experiments has been to minimize as far as possible the time-lag necessary for the different layers of cells to reach the same stage of water uptake. The thicker the discs the greater will be the lag and the curve for the uptake more difficult to interpret, consisting in the case of a thick disc of a series of curves superimposed on one another, due to the time taken for the water to reach the inner layers of cells. The ideal experimental disc would be one layer of cells thick, but this cannot be obtained in practice without risk of injury by cutting.

The obvious way out of the difficulty was therefore to select discs thin enough to approach the ideal condition and yet sufficiently thick to contain more than one layer of uninjured cells.

The discs used were 0.5 mm. in thickness and consisted of four to five layers of cells. Steward (6) has recently pointed out the importance of aeration in maintaining full activity of absorption of the cells of potato tubers. In the experiments here described the discs were left overnight in a thin layer of distilled water or hypotonic solutions, through which air was bubbled. After twelve to fifteen hours' immersion the discs were quite stiff, and on microscopical examination the cells appeared normal and apparently had not lost any of their contents. A slight superficial browning similar to that noted by Steward was observed.

In a preliminary experiment the discs, after being left overnight as described, were then placed in cane-sugar solutions of different plasmolysing strengths, and the change in weight observed. The curves are presented in Fig. 10 and show clearly that the cells have not lost their semi-permeability.

In another experiment, mentioned elsewhere in this paper, the discs were dried for fifteen minutes over calcium chloride before being placed in plasmolysing solutions. The results, as presented graphically in Fig. 13, show that here also the semipermeability of the cell membranes has not been destroyed by the previous treatment; apparently the thickness selected was satisfactory.

Time of drying. The time of drying over calcium chloride was not arbitrarily fixed but was decided after preliminary tests of water loss and subsequent recovery for varying periods over calcium chloride. To obtain the best results two factors had to be considered: (1) to prevent any possible injurious effect on the protoplasm caused by excessive loss of water, (2) to obtain as large a range as possible over which to follow the recovery.

The curves of water loss after varying periods of drying over calcium chloride, and the subsequent recovery in water during a period of five minutes, are shown in Fig. 1. The ordinates represent the actual weights of the tissue and along the abscissae are measured the times of drying and

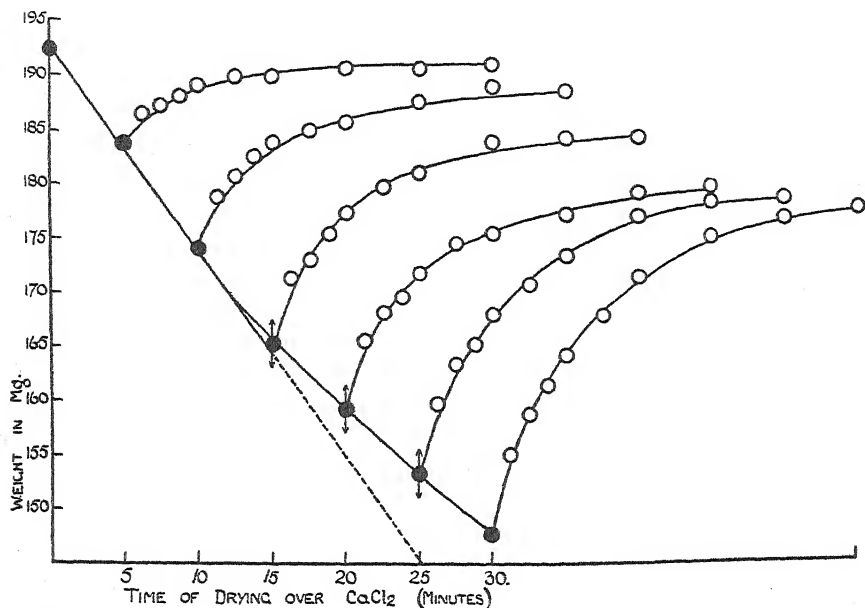


FIG. 1. Uptake of water after varying periods of drying of discs. The range of the standard error is shown. The time scale for uptake of water is five times that for water loss.

of uptake of water during recovery. It is to be noted that the time scale for the curves of water uptake is five times that for the curve of drying. From the descending curve 15 mins. drying was found to satisfy both these conditions. The departure from rectilinearity up to this point is less than one milligram and amounts to less than the standard error of the experiment, and the water deficit is sufficiently great to enable the curve of recovery to be constructed with considerable accuracy. After this period, however, the rate of loss of water over calcium chloride progressively declines. The recovery curves are all of the same type, but become progressively steeper the longer the time of drying. It is seen that recovery is never complete within the time over which weighings were made, but after the period of five minutes the rate of uptake has become very small. From these curves it is clear that the rate of uptake of water is a function of the water deficit induced by drying.

Effect of ions on the uptake of water. The uptake curves in water are presented graphically in Figs. 2 to 9. Fig. 2 represents the uptake after KCl treatment and the corresponding controls. The three points for

each treatment were obtained on three consecutive days with discs cut from the same tuber; the agreement is very striking, as is also the difference in the course of uptake after the two treatments. The final points differ in the two cases, and invariably it is found in these experiments that the final weight of the tissues treated with KCl is very near the original weight before drying, whereas after treatment in distilled water complete recovery does not take place. Fig. 3 shows the uptake curves for KCl, NaCl, and $MgCl_2$ with their controls. The KCl curve is based on ten results, five on each day, whereas for the remaining treatments the curves obtained on two consecutive days are given, and are each based on ten replicates. The KCl results confirm those given in Fig. 2. The agreement between the NaCl curves is not so good, but in each case the Na curve is above the corresponding control for that day. The $MgCl_2$ curves agree well and are considerably below the controls, further curves for $MgCl_2$ are given in Fig. 4 for two consecutive days and confirm the previous result. Fig. 5 shows the effect of NH_4Cl and the curve is well above the control; further curves for NH_4Cl are given in Figs. 6 and 7, and here, although above the controls, the effect is not so marked. In addition, Fig. 6 presents another result for $MgCl_2$ which is again below the control, while Fig. 7 gives the effect of $CaCl_2$. Three other curves for $CaCl_2$ are given in Fig. 2; the results are not in good agreement, but since the salt used in this case was not pure, the results have been given merely in confirmation of those in Fig. 7, in which the pure salt was used. All the results given so far were obtained with potato.

A few experiments were carried out with carrots, since the carrot contains no starch, and a possible effect of starch on the uptake curves was borne in mind. The time of drying was considerably longer (50 mins.) than in the case of the potato (15 mins.), and the water deficit was therefore greater. The results of these experiments are presented graphically in Figs. 8 and 9. Fig. 8 shows the effects of previous soaking in KCl, $MgCl_2$, and D.W., and the replicates of two consecutive days are given. The agreement between the experiments is good, and the results show that the effect of KCl is similar to that in the potato, while the $MgCl_2$, although below the control at first, crosses over and eventually reaches a higher level. Fig. 9 gives the results for two consecutive days with NaCl and $CaCl_2$; the NaCl curve represents the average value of ten replicates done on one day, whereas the remainder are the average values for sets of five replicates in the two experiments.

Reviewing the results as a whole, it is seen that the monovalent ions K^+ , Na^+ , NH_4^+ give curves which lie above these for distilled water in the order given, whereas the bivalent ions Mg^{++} , Ca^{++} , in general lie below. Substantially the same results were obtained with potato and carrot, although the effect on carrot appears less in the graphs, but it should be realized

that the units of the ordinates in the case of the carrot are twice as great as in the graphs for potatoes, so that the absolute differences in weight of water taken up are commensurate.

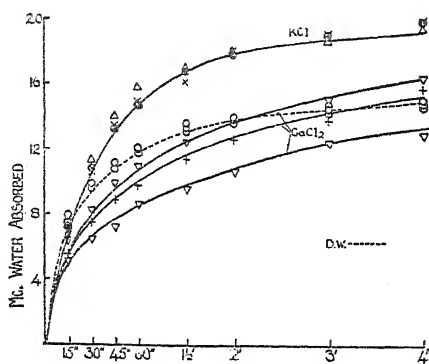


FIG. 2.

FIG. 2. Uptake after treatment with KCl, CaCl_2 and control. The time is given in seconds and minutes. There are three sets of points for each solution. (Potato).

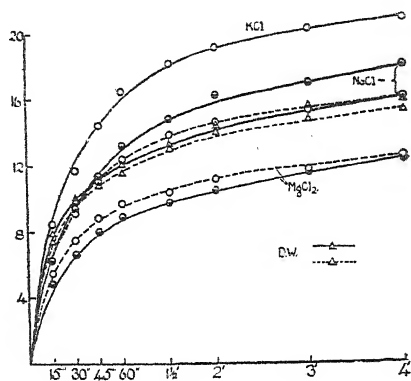


FIG. 3.

FIG. 3. Uptake after treatment with KCl, NaCl (2 experiments) MgCl_2 (2 experiments) and two controls. (Potato).

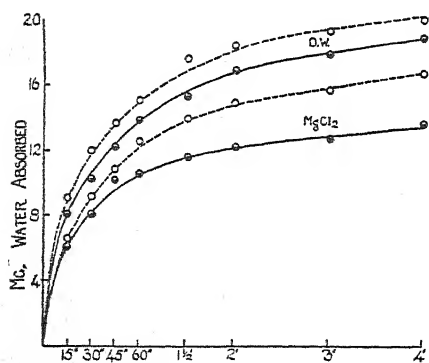


FIG. 4.

FIG. 4. Uptake after treatment with MgCl_2 (2 experiments), and controls. (Potato).

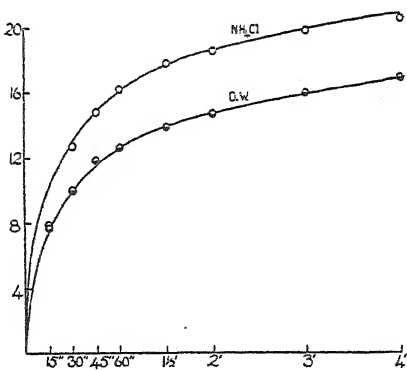


FIG. 5.

FIG. 5. Uptake after treatment with NH_4Cl and control. (Potato).

Statistical analysis of results. It is essential in a quantitative investigation such as the one under review that the validity of the results should be statistically examined. Since consecutive weighings on the same disc have of necessity been used it is not possible to apply the analysis of variance to the data. Three tests, however, have been applied: (1) The average amount of water taken up has been calculated for ten replicates by averaging the series of weighings for each disc for the treatments tested.

From the sets of ten totals thus obtained the means were calculated, and the significance of the difference between the means estimated by Student's method. This test showed whether the average amounts of water taken up

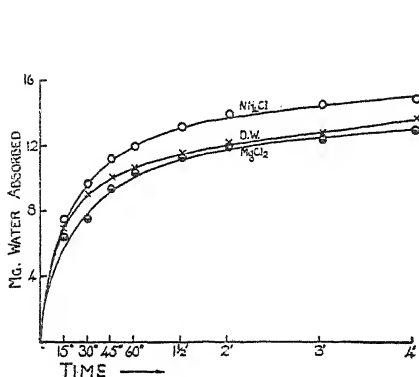


FIG. 6.

FIG. 6. Uptake after treatment with NH_4Cl , MgCl_2 and control. (Potato.)

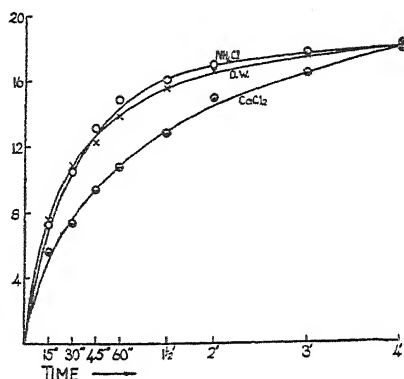


FIG. 7.

FIG. 7. Uptake after treatment with NH_4Cl , CaCl_2 and control. (Potato.)

in a given time were significantly different, but could not show whether the forms of the curves were identical or not. To test this point the following method was used. (2) From the series of weighings it was determined how long after zero time a given proportion of the final amount of water had entered the disc. Sets of ten intervals of time were thus obtained for each treatment, and again the differences of the means were tested by Student's method. A significant difference indicated that the shapes of the curves were actually different. (3) The third method applied was to estimate the rate of uptake of water over corresponding intervals of time in the two treatments, and thus determining on each point of the curve of uptake whether the rates differed significantly with the treatments.

(1) To estimate the experimental errors one experiment was selected at random. The curves are represented in Fig. 4, and the data given in Table I. The sum of squares have been estimated from the values for weights of individual discs at the times stated. The total variance has therefore been estimated from 81 degrees of freedom. The sums of squares in Table I show that the variance of the ten replicates is substantially constant and independent of the time elapsing after beginning of the water uptake. One figure for the standard error may therefore be legitimately used.

The test was applied to results for CaCl_2 and NH_4Cl . The NH_4Cl figures were derived from three separate experiments, the results of which are shown graphically in Figs. 5 and 6. In making the test the figures were combined and the value of t was found to be 4.4 ($n = 54$). The value

of t corresponding to $P = 0.01$ is 2.57; the significance of the result is therefore beyond doubt. A similar test for the results with calcium chloride from one experiment (Fig. 7) gave a value of $P = 2.17$ which is significant. ($P = 0.05$, $t = 2.10$). The test therefore shows that in these two cases the average amount of water taken up was significantly different from the control values.

TABLE I.

Time (secs.)	Distilled water.		MgCl ₂ .	
	Mean.	$S(x - \bar{x})^2$	Mean.	$S(x - \bar{x})^2$
0	116.0	227.7	112.3	209.6
15	124.0	186.7	118.3	227.6
30	126.2	198.1	120.3	196.1
45	128.2	202.6	122.5	204.5
60	129.8	202.5	122.8	216.0
90	131.3	183.1	124.0	222.0
120	132.9	213.4	124.5	206.5
180	133.9	215.7	125.0	219.2
240	135.0	219.7	125.9	216.9
	128.6		121.7	

The mean values for the two treatments derived from replicates of ten with their standard errors were found to be:—

Distilled water	128.6 ± 1.51
MgCl ₂	121.7 ± 1.53

(2) The second test was applied only in the case of Ca and Mg, since in the graphs these are the only curves which apparently differ in shape from the controls. The data used are those represented in Figs. 7 and 4. The value for Mg is quite insignificant ($P = 0.3$), but for calcium the value of t was 8.9 which leaves no doubt as to the significance of the difference in form of the Ca curve from the control.

(3) The third test mentioned above was applied to the K, Na, Ca, and Mg results. The results of the test are shown in Tables II and III. Significant results are italicized.

TABLE II.

Time (secs.)	Rate of uptake of water in milligrams per minute.		Difference in rate.	t .
	KCl.	D.W.		
15	22.08	20.17	+1.91	1.76
30	12.01	7.12	+4.89	3.56
45	7.79	3.68	+4.11	4.37
60	5.62	3.17	+2.44	4.72
120	2.15	1.14	+1.01	3.65
180	0.91	0.88	+0.03	1.08

Number of degrees of freedom = 18.

5 % probability value of t ($P = 0.05$)	$t = 2.10$
1 % " " " ($P = 0.01$)	$t = 2.87$

Results of a similar test for Na and Mg derived from the data in Fig. 3 gave the values in Table III.

TABLE III.

(Significant differences are italicized and the probabilities given in brackets.
Number of degrees of freedom = 18.)

Time (secs.).	Rate of uptake mg. per min.			Difference in rate.	
	NaCl.	MgCl ₂ .	D.W.	NaCl-D.W.	MgCl ₂ -D.W.
15	18.3	13.2	20.1	-1.80 (<i>P</i> = 0.05)	-6.90 (<i>P</i> < 0.01)
30	10.3	6.3	8.2	+2.10 (<i>P</i> = 0.05)	-1.90
45	8.2	4.6	4.2	+4.00 (<i>P</i> < 0.02)	+0.40
60	5.8	3.25	3.25	+2.55 (<i>P</i> < 0.01)	0.00
120	1.93	1.4	1.78	+0.15	-0.38
180	0.95	1.0	0.88	+0.07	+0.12

The results in the Table indicate that in the case of Na the first point at which the Na curve is below the control is just significant, the remainder up to 60 secs. are very significantly positive. In the case of MgCl₂ only the first point is significantly different, showing that the slopes of the curve of uptake after this point are similar, for which reason the second test on the difference of forms of the curve failed in the case of Mg. It will be seen that significant results have been obtained with all the statistical tests made.

The Form of the Uptake Curve.

The problem of determining the differences in the rate of uptake after various treatments would have been much simplified had it been possible to find some simple function of time in which to express the changes in rate during the recovery. The simplest assumption possible is that the rate of uptake is directly proportional to the water deficit, or in other words, that:

$$dx/dt = k(A - x)$$

where x represents the weight of water in the tissues at time t and A the weight of water in the fully turgid state. The difficulty of applying the equation arises from the uncertainty as to the value to be attributed to A . Since recovery was found to be incomplete A could not be assumed to be the original weight before partial desiccation, nor could A be taken as the weight at the end of the experiment, since it was experimentally shown that the discs did not attain a constant weight. It was found that the integrated form of the equation would not fit the experimental data with any accuracy, and in this respect the results in these experiments do not agree with those obtained by McCutcheon and Lucke (3). Other functions were tried but none gave satisfactory agreement with the experimental results. For this reason the third statistical test mentioned above was based on the actual rates of uptake at each point on the curves. This rate

was determined by calculating the relative rate of increase over periods of half a minute or one minute respectively, and multiplying this by the weight of water in the tissues at the half period point. Thus: relative

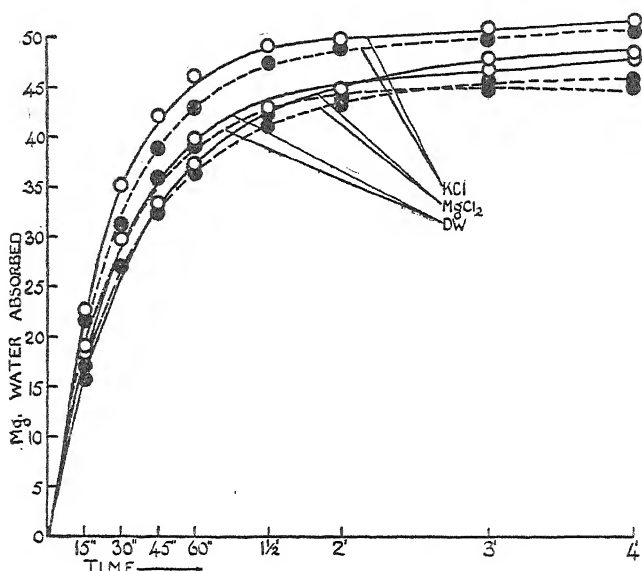


FIG. 8. Uptake after treatment with KCl, MgCl₂ (2 experiments) and controls. (Carrot.)

rate = $1/x \times dx/dt$: therefore dx/dt absolute rate of increase = x times the relative rate.

The following table shows values of the rate for the various treatments.

TABLE IV.

Time (secs.).	Rates of uptake of water (mg. per min.)					
	KCl.	NH ₄ Cl.	NaCl.	Control.	MgCl ₂ .	CaCl ₂ .
15	20.00	17.88	17.16	18.04	14.00	13.82
30	11.63	10.39	9.32	6.49	6.38	5.83
45	7.64	6.18	6.24	4.16	4.35	4.77
60	4.00	3.10	3.74	2.80	2.37	3.37
90	2.47	1.94	2.47	2.05	1.78	2.73
120	1.30	0.98	1.17	1.01	0.84	1.76
180	0.60	0.48	0.78	0.65	0.67	1.28

The figures in the table are the averages of the rates for each ion for all the experimental data available. The values for the monovalent ions are generally above the control values, and the difference of the rates declines in the order given in the table. The bivalent ions are at first below the control values, but later become either equal or higher, which corresponds with the crossing over of the uptake curves as seen in the graphs. In the case of the carrot the results for K', NH₄', Na', and Ca'' were

the same as for the potato, but the magnesium showed no consistent difference from the control.

The data presented above indicate a real difference in uptake rate as the result of soaking in hypotonic solutions of various metallic salts.

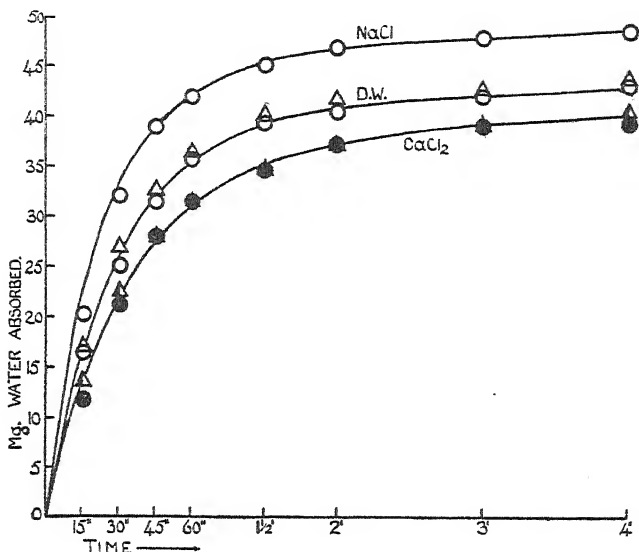


FIG. 9. Uptake after treatment with NaCl, CaCl₂ and control. (Carrot.)

The Suction Pressure of the Potato Discs.

Before ascribing the foregoing results to a change in permeability, it is necessary to show that the previous treatment had not differentially altered the osmotic pressure within the cells as a result of diffusion inwards of the salts, or exosmosis of the cell contents.

To determine approximately the suction pressure of the discs the following method was used :

Standard discs, cut directly from potato tubers which had been kept for some time in a saturated atmosphere were placed in sucrose solutions of known osmotic pressure derived from the tables of Ursprung and Blum (7). The loss in weight of the discs in one series of experiments is shown graphically in Fig. 10. The data of percentage loss in weight after 5 minutes immersion for all available experiments are presented in Table V below.

These values (black discs) are presented graphically in Fig. 11 together with the calculated line of closest fit determined from all the data.

The equation for this line is

$$C = 5.079 - 2.257 P \text{ (i)}$$

where C is the percentage loss in weight, and P the osmotic pressure of the sucrose solution. Values of C calculated from this equation are entered in the third column of Table V. The relation of percentage water loss and

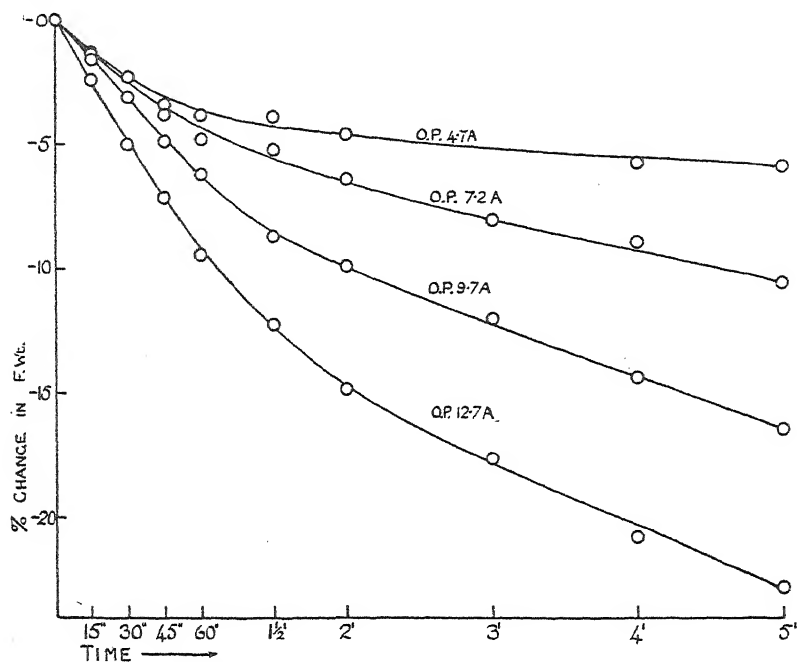


FIG. 10. Water-loss in hypertonic sugar solutions. Discs fully turgid.

TABLE V.

Osmotic pressure (atm.) of solution.	Loss in weight (%) after 5 minutes.	Calculated values.
12.7	22.8	23.5
11.1	20.7	19.9
9.7	{ 16.4 }	16.8
8.4	{ 16.3 }	
7.2	15.7	13.9
5.9	10.4	11.2
4.7	6.9	8.2
	{ 5.8 }	
	{ 6.2 }	5.5
3.5	3.2	2.8

osmotic pressure closely approximates to a linear one. In Fig. 11 (open circles) are also represented the final values of water loss after a considerably longer period of immersion, generally two hours. These values also lie approximately about a line meeting the O.P. axis at about 2 atmospheres. The calculated line of closest fit is drawn, whose equation is

$$C = 4.322 - 2.673 P \text{ (ii).}$$

The fact that neither of these lines passes through the origin shows

that the suction pressure of the tissues was not zero at the time of cutting, in spite of the tuber having been kept in a saturated atmosphere, and the preliminary washing with distilled water. This, no doubt, is due to

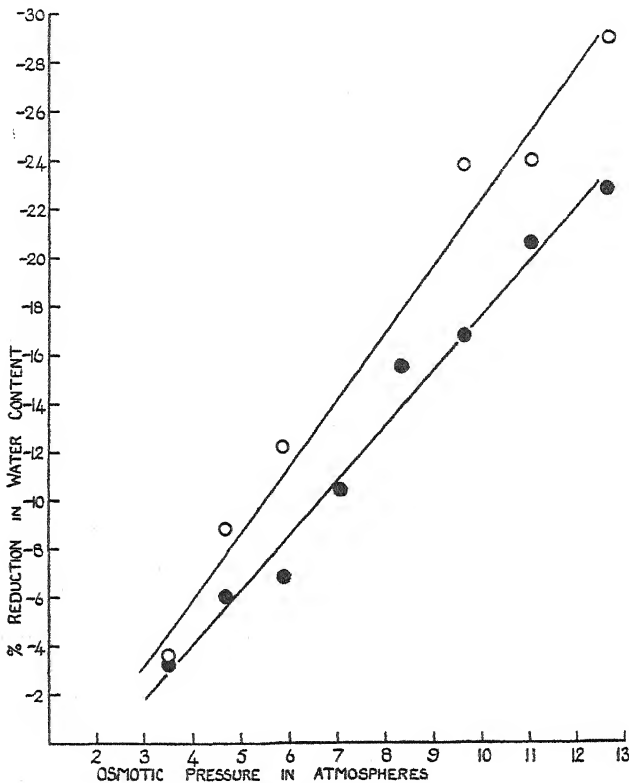


FIG. 11. Relation between reduction in percentage water content for potato discs immersed in sugar solutions of known osmotic pressure. Dots, after five minutes; open circles, after two hours.

a component of opposing pressure to the osmotic pressure represented by the mutual pressures of the turgid cells (see Ernest (1)).

In every experiment the weight of the discs when first cut from the tuber was recorded as well as the weight of the discs after the preliminary drying over calcium chloride. From the percentage reduction in water-content calculated from the data, the corresponding suction pressure has been calculated by the equation (i). The results are presented in Table VI, in which are entered the various values of the suction pressures for discs after preliminary soaking in hypotonic solutions of the salts, together with the corresponding values for the appropriate controls left in distilled water. All available data are recorded.

The mean differences, taking the distilled water as standard, are given

together with their standard errors, and in one case only (NH_4Cl) are these differences statistically significant, and, further, the algebraic sign of the differences bears no relation to the valency of the ions.

TABLE VI.

Suction pressures (atmospheres).							
KCl.	Water.	NH_4Cl .	Water.	CaCl_2 .	Water.	MgCl_2 .	Water.
9.18	9.45	9.00	8.87	9.40	9.45	8.91	9.09
8.91	9.00	10.16	9.36	9.45	9.00	9.72	9.45
9.18	9.45	10.74	10.30	8.95	9.45	8.87	8.87
9.40	9.09	10.56	10.43	9.80	9.36	9.80	10.12
9.45	9.45			10.52	10.30	9.71	9.71
				10.56	10.43		
9.224	9.288	10.115	9.740	9.780	9.667	9.402	9.448
Mean diff.	-0.064		+0.375		+0.113		-0.046
Standard error	± 0.11		± 0.16		± 0.15		± 0.09

The mean value of suction pressure based on the data is 9.58 atmospheres. This value may be compared with a direct determination of the S.P. of the discs after preliminary drying over CaCl_2 . In one series of experiments the discs as cut from a single tuber were first dried for the standard time over CaCl_2 and then immersed in sucrose solutions of known osmotic pressure. After 5 minutes the weights of the discs were determined and the per cent. loss or gain in weight found. The results are shown graphically in Fig. 12. The relation is very nearly linear and the suction pressure of the solution corresponding with no change in weight is found to be 9.2 atmospheres. This value for suction pressure agrees with the calculated values given above, and lies well within the range of values given in Table VI.

A series of experimental results of water uptake in various solutions after preliminary drying are shown graphically in Fig. 13. There is a large increase in the first 15 seconds followed by a fall when the disc of tissue is immersed in a hypertonic solution. This large initial increase may be ascribed in part to the instantaneous uptake of water by wetting of the surface of the dried disc. Experimentally it has been shown that the increase thus obtained is small compared with the uptake in the first fifteen seconds. It is obvious that however rapidly the disc is immersed and dried some water will have been taken up by the cells during the process of drying with blotting paper. Whether this can account for this apparent instantaneous effect cannot be experimentally decided. It is possible, however, to account for this effect in another way. The disc has been dried for fifteen minutes over calcium chloride, water evaporates from the surface cells, the flow of water outwards takes place as a result of the gradient of suction pressure from outside inwards established by evaporation. When the disc is immersed in a hypertonic solution the surface

cells, having a suction pressure higher than the osmotic pressure of the outside solution, take in water, and meanwhile water continues to be withdrawn from the inner cells, thus increasing the suction pressure of the inner

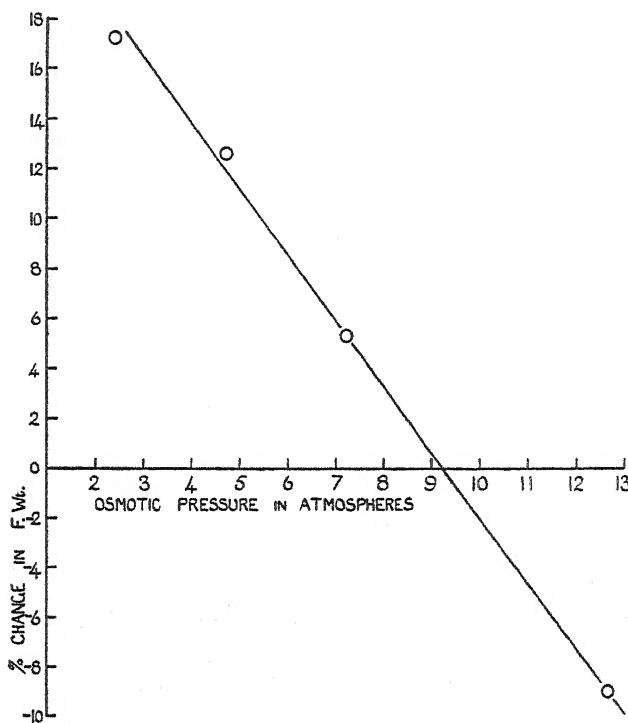


FIG. 12. Relation between percentage change in water content and osmotic pressure of sugar solutions. Discs previously dried over CaCl_2 for 15 minutes.

layers. The suction pressure of the outer cells thus falls, while that of the inner cells rises. A condition is finally reached in which all the cells have the same suction pressure.

If the average suction pressure of the disc be higher than the osmotic pressure of the external solution water will be taken in. When the average suction pressure of the tissues is less than the osmotic pressure of the outside solution water will be withdrawn and a flow outwards results. Again, when the average suction pressure of the disc after the disappearance of the gradient in the tissue is the same as the osmotic pressure of the outside solution, water will neither enter nor leave the disc. All three cases are well shown in Fig. 13.

The Absolute Value of Permeability to Water.

Permeability has been defined earlier in this paper as the mass of water passing per unit area per unit time under unit suction pressure

gradient. From the data presented above it is therefore a simple matter to calculate the absolute permeability to water of the discs used. The relevant factors of area, rate of uptake and suction pressure are all known.

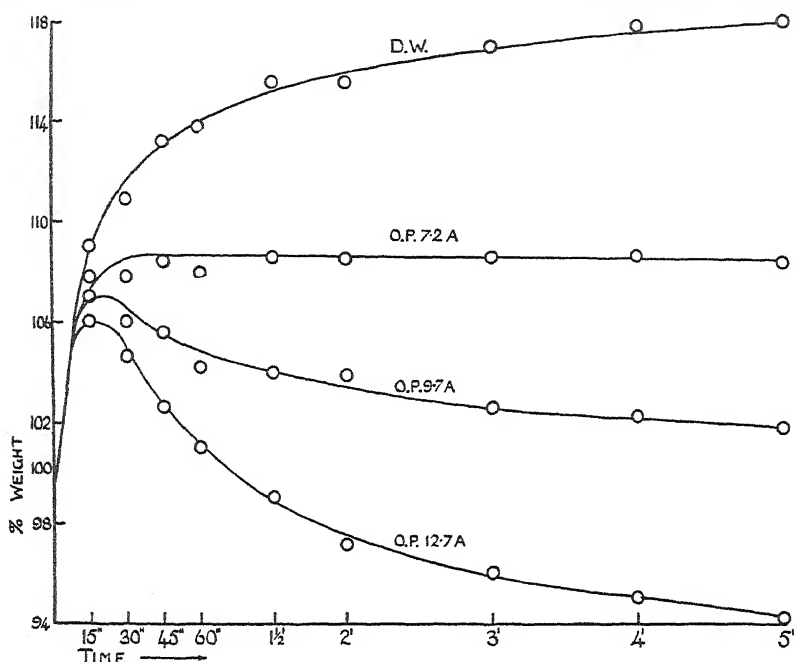


FIG. 13. Curves for water uptake in hypertonic sugar solutions by discs previously dried for 15 minutes over CaCl_2 .

The discs were uniformly cut to a thickness (t) of 5 mm. and diameter (d) 1.8 cm. The total surface of the discs is therefore as follows:

$$\text{Area of surface} = \pi d^2/2 = 5.10 \text{ sq. cm.}$$

$$\text{Area of rim} = \pi dt. = 0.28 \text{ " "}$$

$$\text{Total} = 5.38 \text{ " "}$$

The value of permeability has been calculated for both loss and uptake of water. For the former, the data used were those obtained by immersing discs in solutions of cane sugar of known osmotic pressure. Some of these data are shown graphically in Fig. 10. The linear relation between the percentage loss of water and the osmotic pressure of the external solution has already been indicated. The data in Table V show the relation of loss in weight after five minutes to the O.P., together with the values calculated from the line of closest fit. In the data to be presented later, in which the suction pressure of the disc is calculated at varying times from immersion the assumption has been made that the percentage water deficit of the disc is proportional at all times to the suction pressure. The importance

of this assumption is paramount, so that further evidence on this point may be presented here. From the series of weight curves in various solutions, as shown in Fig. 10, it is possible to calculate the relation between percentage water deficit and external osmotic pressure for each interval of weighing. The equations of the lines of closest fit are given below, and in Table VII are presented the actual and calculated values of percentage water deficit for various solutions and times of immersion.

TABLE VII.

O.P. of external solution (atm.).	Time from immersion of discs (min.)						
	0.5.	1.0.	1.5.	2.	3.	4.	5.
12.7	5.0	9.4	12.2	14.8	17.6	20.8	22.8
11.1	2.7	7.4	10.0	12.0	15.9	18.2	20.7
9.7	4.0	7.3	9.2	10.7	12.2	14.6	16.3
8.4	3.0	4.6	9.1	10.5	12.7	14.0	15.7
7.2	2.3	4.8	5.2	6.4	8.0	8.9	10.4
5.9	1.3	3.1	4.1	5.0	5.5	6.4	6.9
4.7	2.4	3.8	4.4	4.8	5.4	5.9	6.0
3.5	1.2	2.4	2.9	2.3	3.1	2.9	3.2
12.7	5.47	9.64	12.46	14.91	18.05	21.03	23.58
11.1	4.61	8.33	10.71	12.74	15.39	17.85	19.97
9.7	3.87	7.18	9.08	10.84	13.05	15.07	16.81
8.4	3.17	6.12	7.77	9.07	10.89	12.48	13.88
7.2	2.53	5.14	6.46	7.45	8.89	10.10	11.17
5.9	1.84	4.07	5.04	5.69	6.72	7.51	8.23
4.7	1.20	3.09	3.73	4.06	4.72	5.13	5.55
3.5	0.56	2.11	2.42	2.43	2.72	2.74	2.82

Equations to lines of closest fit.

y = % water deficit; x = osmotic pressure of solution.

0.5 mins. after immersion:	$y = 0.534x - 1.313$
1.0 " " "	$y = 0.818x - 0.752$
1.5 " " "	$y = 1.091x - 1.396$
2 " " "	$y = 1.357x - 2.320$
3 " " "	$y = 1.667x - 3.117$
4 " " "	$y = 1.988x - 4.215$
5 " " "	$y = 2.257x - 5.079$

Although large discrepancies appear, yet there is no evidence of any systematic departure from a linear relation. Assuming then that a linear relation between suction pressure and water deficit holds, from the weights of discs at varying times after immersion the suction pressure at each point can be estimated. For this purpose the following equation was used:

$$y = 2.257x - 5.079,$$

y being the percentage water deficit, and x the suction pressure of the disc. From these values the average suction pressure during the interval was calculated from the expression

$$\bar{S} = \frac{S_1 - S_2}{\log S_1 - \log S_2},$$

where \bar{S} is the average suction pressure and S_1 and S_2 the suction pressures at two different times. The effective suction pressure is then the difference between this value and osmotic pressure of the external solution. Since the loss of water in the interval of time is known, the permeability can be directly obtained by dividing the water loss by the effective suction pressure. The result of the calculation gives the rate for each disc of loss in mg. for each interval (15, 30, or 60 secs.). To convert this figure to absolute values of permeability it must be multiplied by a conversion factor as follows:

If p = water loss in mg. per min. per disc, then for the absolute value P

$$P = p \times \frac{60 \times 10^4}{5.38 \times 10^3} = 112 \times p \text{ gm. per sq. metre per hour.}$$

TABLE VIII.

Osmotic Pressure of External Solution 12.7 Atmospheres.

Time from immersion in min. and sec.	Wt. of disc in mg.	S.P.	Av. S.P.	Eff. S.P.	Water lost (mg. per disc).	P gm. per sq. metre per hour per atm.
0	152.2	2.21	2.71	9.99	3.6	157
15 secs.	148.6	3.28				
30 "	144.6	4.44				
45 "	141.4	5.38	4.90	7.80	3.2	184
1 min.	137.9	6.41	5.88	6.82	3.5	230
1.5 "	133.6	7.66	7.02	5.68	4.3	170
2 "	129.7	8.82	8.23	4.47	3.9	196
3 "	125.4	10.07	9.45	3.25	4.3	149
Time interval (min.).	O.P. = 12 atm. P .	O.P. = 9.7 atm. P .		O.P. = 7.2 atm. P .		
0-1	183	162		194		
0.5-1.5	181	185		167		
1-2	178	157		103		
2-3	149	133		151		
Mean	173	160		154		

In the above table are given the actual values of permeability determined from each pair of consecutive weighing of discs immersed in a cane-sugar solution of O.P. 12.7 atmospheres. The values, although somewhat irregular, are all of the same order, and, further, show no very regular drift with time. In the second part of the table are given values of P calculated for consecutive minute periods, for three different cane-sugar solutions of

the O.P. stated. Again, the values of P are of the same order in each case. There appears to be some fall in the mean values of P as the solutions become more dilute, but this difference is by no means concordant in the successive periods and may be regarded as experimental error. From the nature of the method large variations in the value of P may be expected.

The values of P so far given may be compared with those obtained from the uptake values, derived from experiments in which the discs were partially dried and then allowed to take up water. Two types of experimental data have been used.

(1) Uptake of water after preliminary drying for varying periods over CaCl_2 . The original S.P. of the disc was calculated from the percentage water deficit after drying in the usual way. A further difficulty, however, presents itself, in consequence of the fact that the disc, even after prolonged immersion in water does not recover the original fresh weight, but invariably reaches only a lower value. In order to calculate the suction pressures at intermediate points the value after five minutes was assumed to be zero and the intermediate values calculated as proportional to the deficit in water content.

TABLE IX.

Time intervals (min.).	Values of P for different times of drying over CaCl_2 .			
	15'.	20'.	25'.	30'.
0-1	179	185	143	148
0.5-1.5	164	143	119	117
1-2	144	110	132	133
2-3	183	77	180	125
Means	168	129	144	131

The values of permeability thus obtained are entered in the above table. The order of permeability is similar to that obtained for loss of water. The mean values show a fall for longer periods of drying, but the figures are somewhat irregular and therefore cannot be accepted as definitive. Further, a tendency is apparent for the values to fall during recovery of the disc. Again the values are too irregular to establish this with certainty.

(2) A further series of permeability values for uptake of water are given in Table X.

TABLE X.

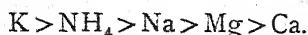
Time intervals (min.).	Values of P .	
0-1	287	270
0.5-1.5	197	185
1-2	138	136
2-3	103	112
Mean	181	176

These values were obtained from a series of control discs, dried for fifteen minutes over CaCl_2 , and then allowed to take up water. The values are shown graphically in Fig. 3 above. Values of permeability of the same order are again obtained. The mean values compare favourably with those in Table IX for discs dried for fifteen minutes. Further, in this series there is considerable evidence of a fall in permeability as recovery proceeds. This is no doubt due to the phenomenon already noted above, namely, after the period of drying there is established in the disc a gradient of suction pressure from the surface inwards. The values used in the calculation of permeability are derived from the average S.P. of the discs. The S.P. of the outermost cells will therefore be underestimated, and the permeability figure obtained will in consequence be too high. A further consideration seems to be pertinent in this connexion. Water entering the disc at first has to pass only one layer of protoplasm, whereas later when water is penetrating the inner layers of cells a larger number of protoplasmic layers has to be traversed. For this reason also the permeability values should fall. In the case discussed above of water loss in cane-sugar solutions there is present no gradient of S.P. within the disc, and water movement within the whole disc is involved from the start. In this case, therefore (Table VIII), there is a much smaller fall in the permeability values after immersion. The case in which the disc is dried before immersion has already been dealt with.

DISCUSSION.

The results described in this paper cannot be directly compared with those dealt with by Stiles. The uptake of water in the experiments of Stiles and Jörgensen (8) occupied some hours, and did not even approximately attain equilibrium, whereas the uptake recorded in this work was nearly complete after a few minutes. In this respect these results are comparable with those obtained by Huber and Höfler and de Haan. It is therefore open to doubt whether in the experiments of Stiles and Jörgensen the protoplasmic permeability was controlling the rate of uptake of water. Nevertheless it is of interest to note that the high temperature coefficient of water uptake for potato tuber found by Stiles and Jörgensen ($Q_{10} = 2.7-3.0$) have been corroborated in this work,¹ and also by de Haan ($Q_{10} = 2.7$).

As to the specific effects of the ions studied, the series of decreasing permeability found is as follows:



¹ Exigencies of space have prevented the inclusion of these data. The average value found was $Q_{10} = 2.9$. Full details may be seen in the Library of London University. (Thesis for M.Sc. degree: 'The Effects of Metallic Ions on the Permeability of Cells to Water', by E. C. D. Baptiste.)

Except for the reversal of K and NH_4 the results conform with the Hofmeister series. The results given by de Haan for deplasmolysis in cane sugar form a series $\text{Na} > \text{K} > \text{Mg} > \text{Ca}$: the values for Na and K are nearly identical, also those for Mg and Ca. The specific effects of the ions agree therefore in the sense that monovalent ions are associated with higher permeability than bivalent ions. A similar result was found with *Arbacia* eggs by McCutcheon and Lucke (5). The discrepancies in actual order are of no great account. Different types of tissue were used, and, as Michaelis (9) points out in this connexion, 'in different cases different kinds of effects become manifest, not even the series of ions always remaining the same'.

De Haan gives an excellent review of current theories on the specific action of ions. He inclines to the view that the chief effect is due to variation in the rate of swelling of the protoplasm. Support of this view is suggested by the variation in magnitude of the permeability during the course of deplasmolysis: the gradual increase in permeability is ascribed to increase in imbibition of the protoplasm during the recovery process. Higher temperatures also accelerate this change, due to the high temperature coefficient of imbibition. In the experiments here described no such rise in permeability was noted, indeed a fall occurred, due to quite other causes.

The absolute magnitude of permeability as calculated from the present data is of the order of 150–200 c.c. per sq. metre, per hour, per atmosphere S.P. This value is higher than those given by Huber and Höfler, who found for *Salvinia* a value in the same units of 33 c.c., though for *Vallisneria* higher, and for *Majanthemum* lower values were obtained. De Haan with *Allium cepa* obtained values from 7–56 c.c. per sq. metre, per hour, per atmosphere, the actual value depending on the stage of deplasmolysis and the agents used. Huber and Höfler, moreover, found that the permeability is greater for loss than for uptake of water. No such variation has been noted in these experiments.

The very high resistance to water movement has been commented on in both the papers cited above, and the relation of this to the general problems of water economy is discussed. One remark in this connexion will suffice: the greater part of resistance to water movement may be located not in the vessels, but at the surfaces of the mesophyll cells of the leaf. Experimental investigation of this point is now in progress.

In conclusion, it may be stated that the method employed in this work of increasing the suction pressure by controlled evaporation has some advantage over the method of plasmolysis, since possible injurious effects of plasmolysis are avoided. On the other hand, the disadvantage of working with a cell complex rather than single cells is patent.

SUMMARY.

1. Uniform discs of potato tuber and carrot were used, and the uptake of water was followed by periodical weighings at intervals of one minute or less.

2. The discs were soaked overnight in hypotonic solutions of chlorides of K, Na, NH_4 , Mg, Ca, conditions of good aeration being maintained.

3. The suction pressure of the discs was then raised to a known uniform level by controlled evaporation over CaCl_2 , and the uptake of water was followed for five minutes after immersion in distilled water.

4. The effects of the cations on permeability followed the series $\text{K} > \text{NH}_4 > \text{Na} > \text{Control} > \text{Mg} > \text{Ca}$. The differences in rate of uptake are shown to be statistically significant.

5. The variation in rate of uptake is shown to be due to permeability changes, for no differences in suction pressure result from the previous exposure to the salt solutions.

6. The absolute permeability of the discs to water is calculated. A value of 150–200 c.c. per sq. metre, per hour, per atmosphere is obtained. The same value holds both for uptake and for loss of water.

In conclusion, the author wishes to record his indebtedness to Professor V. H. Blackman for much valuable criticism, and to Dr. F. G. Gregory, at whose suggestion the work was undertaken, for help during the work and in preparation of this paper.

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Observations on the Occurrence of Air in Conducting Tracts.

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SINCE the question of the occurrence of bubbles of air¹ or vapour in the functional layers of the wood of trees in nature still appears to remain open, and in view of the special significance of such occurrence to the cohesion theory of conduction, it appeared desirable to attempt to devise some new methods of testing for the presence of air or vapour in the tracts of transpiring woody plants. Some simple tests have therefore been devised which are readily applied in the field with very little apparatus. By their use it can be shown that bubbles are present at certain times and under certain conditions, but that at other times and during other conditions they disappear.

The tests are based on the observation that if a few square cm. of the bark of some woody branches be very carefully removed so as to expose uninjured the surface of the wood, the latter appears to be homogeneously translucent and of a uniformly, slightly dark, greyish or yellowish colour, provided that no bubbles are present in the superficial conducting tracts. If, however, the surface of the wood be pricked with a needle or the point of a scalpel, or if any minute incisions be made in it, air is immediately admitted to the injured superficial tracts, and the tracts containing air can then be easily distinguished from the rest, even with the naked eye, by the presence of a lighter-coloured streak extending upwards and downwards from the incision or prick. The light streak produced is very definite and unmistakable, being given most distinctly among the species studied so far by the Elder (*Sambucus nigra*), the Lilac (*Syringa*), Ivy (*Hedera helix*), the Hawthorn (*Crataegus oxyacantha*), and the Ash (*Fraxinus*). It is evidently due to the total reflection that takes place from the inner surfaces of the tracts to which air is admitted, and gives the appearance of a decrease in transparency of the affected areas. Further it is obviously due to the

¹ For convenience the bubbles are referred to throughout as 'air' although they must consist largely of water-vapour, their composition no doubt varying from time to time.

penetration of air, since the test is only given when the contents of the tracts would be expected to be at less than atmospheric pressure so that air would be drawn in, and not if the branch be first cut off under water and well supplied with water for an hour or so before applying the test. It is evident, then, that if air is naturally present in the superficial tracts, it can be seen as lighter streaks, provided that some of the superficial wood still contains water to act as a standard for comparison, and air is not present throughout all the superficial tracts. Thus if some bark be carefully removed from an experimental branch and streaks are visible air is definitely present, but if no streaks are visible air may be either universally present or universally absent. To decide which of these is the case it is only necessary to perform the 'puncture test' which consists in pricking the surface of the wood with a needle. If nothing happens on puncturing air must be universally present, but if streaks are then produced there can have been no air present originally. For convenience the examination for naturally occurring streaks will be referred to as the 'streak test', and the examination for streaks resulting from puncturing will be referred to as the 'puncture test'. Thus, if the streak test be positive, some air is present according to the relative proportions of the light and dark areas of the wood surface; if the streak test be negative and the puncture test positive, no air is present, and if both tests be negative the tracts are full of air. These conclusions, however, are only valid if it can also be proved that the pressure in the tracts is sufficiently reduced to cause air to be drawn in on making the puncture, otherwise the puncture test will be negative in all cases and fails as a differentiating agent. To prove that the tract pressure is sufficiently low to allow the puncture test to work, the 'indian ink test' and the 'eosin test' are applied.

The indian ink test and the eosin test consist in performing experiments exactly similar to the puncture test, but with a needle or scalpel which has been very lightly dipped into indian ink or eosin. If the tract pressure be below atmospheric the ink or the eosin is immediately drawn into the puncture. This being so, it is usually safe to assume that air would be drawn in in the same way. Occasionally it appears that if the pressure in the tracts is only just below that of the atmosphere, eosin may be slowly drawn in but not air, on account of the surface-tension effect at the puncture (cf. p. 377). This condition, however, can be detected by the very sluggish intake of eosin. The streak and puncture tests can therefore only be taken together as a test for the original presence of air at times when the indian ink and the eosin tests are distinctly positive, but in the writer's experience this appears to be almost invariably.

A confirmatory test, which can be applied in the case of naturally occurring streaks, is the 'water test'. In this the puncture test is performed with a scalpel or needle point which has been dipped into water.

If applied to the darker areas of the wood showing naturally occurring streaks no change is observable, but if applied to the streaks themselves the punctured streaks disappear through the absorption of water into the air-containing tracts. In some woods (e.g. *Ulmus*, *Corylus*, &c.) the presence of localized fibres containing air leads to appearances very similar to those produced by the streaks due to air in the tracheae. These fibre-streaks can, however, readily be distinguished from the air-containing tracheal streaks by applying the water test to the streaks. If the streaks be due to tracheae containing air, the result of the water test is positive and the streaks disappear, but if due to fibres the streaks remain. The indian ink and the eosin tests, moreover, also give positive results with tracheal streaks, and negative results with fibre-streaks, as the ink or dye penetrates into the tracheae but not into the fibres. If eosin and water, &c., do not enter the streaks as a result of reduced pressure, it should also be determined whether eosin solution can be taken up by capillarity. To test this, two transverse slits are made in the surface of the wood, one above the other, and 4-5 cm. apart, and the dye applied at the lower slit. The upper slit allows the escape of air displaced by the rising column of dye in the tracts. If the dye does not enter the streaks in these circumstances, the streaks are only fibre-streaks and must be ignored.

In some cases, as with *Alnus*, *Corylus*, *Acer*, and *Ulmus*, it is sometimes found that the bared surface of the wood appears uniform, and that owing to the particular nature of the wood, both the puncture test and the water test fail to give any definite result, in spite of the fact that the ink and eosin tests are definitely positive. This must mean that in these cases the optical properties of the wood are such that the relative amounts of light reflected from air-containing and water-containing tracts do not allow these two to be distinguished. Similar difficulties occur also at such times of the year (usually about November to March) as the most superficial of the larger conducting elements are overlaid by a layer of relatively non-conducting autumn wood. Since the tests naturally give the most definite and most easily visible results when performed on the larger conducting elements, such as the vessels of the spring wood, the results are not as conclusive or satisfactory when the last layer containing the larger elements is obscured by a relatively non-translucent layer of very small conducting elements, starch-containing wood parenchyma, and fibres. In these cases, as in various other cases of uncertainty with the less amenable types of wood, further evidence can be obtained by noting the exact behaviour of the penetrating ink or eosin column when the ink and eosin tests are applied. It can be readily observed in cases where streaks are naturally present, i.e. some tracts contain air and some do not, that ink and eosin penetrate very differently into the air-containing and water-containing tracts. If the tracts contain air there is usually a relatively

small amount of penetration of ink or dye, such as there is being due to reduced air-pressure and to capillarity. The travel in these cases is relatively slow and soon comes to a stop, the penetrating column reaching a height of only a few centimetres. When, however, the tracts contain water under a considerably reduced pressure or tension, the reagent is drawn in so rapidly that it is often impossible to follow the top of the column with the eye. The whole of the experimental drop on the tip of the scalpel is very quickly drawn in (especially with eosin, which does not clog the tracts as the ink is inclined to do), and the tail of the column disappears, leaving the tract full of air near the puncture. In other cases, when the tracts are normally full of water under tension, a series of little bubbles may be drawn into the tracts alternating with short columns of the dye or ink. The distance penetrated, as well as the speed of penetration, is usually much greater when the tracts contain water rather than air, since, by removing a further area of bark higher up, the penetrated reagent can in these cases be found 30-40 cm. above the level of the puncture within a second or so of applying the test. These facts are frequently of use in discriminating between tracts containing water and those containing air.

Penetration of ink or dye may therefore indicate either (*a*) tracts full of water under reduced pressure or tension, if the penetration be rapid and continuous, or (*b*) tracts full of air, if the penetration soon ceases. Lack of penetration may indicate either (*a*) tracts full of water at atmospheric pressure, or (*b*) tracts full of water under pressure greater than atmospheric pressure.

The extent of the capillarity-effects in air-filled tracts may be investigated for purposes of comparison by cutting two horizontal, transverse grooves in the surface of the wood about 10 cm. apart, one vertically above the other. Blotting paper can be applied to the lower groove to empty the superficial tracts between the grooves of water, and the eosin or ink test can then be applied to the wood between the grooves or to the lower groove. The rise of the ink or dye in the air-filled tracts is noted and compared with that in the tracts not artificially emptied in this way.

In cases where the conducting wood is obscured by layers of fibres or winter wood which is relatively difficult to work on, results may also be obtained by shaving off the superficial layers of the wood with a razor from above downwards with a stream of water flowing over the surface and applying the tests to the underlying layers of spring wood. This method does not give such definite results, however, and involves the necessity of more apparatus. There is, moreover, the possibility of damaging the deeper wood during the removal of the upper layers. In practice therefore it has been found better, as far as possible, to utilize trees giving definite results with the superficial wood, and only to use such a method when absolutely necessary for completing series at different times of the year.

Apparatus and Technique.

The apparatus normally required is extremely simple. It consists of three small bottles containing respectively a few c.c. of indian ink, 0.5 per cent. eosin solution, and water, two scalpels, one pair of fine scissors, forceps, a needle, and sometimes a hand-lens, though this is actually seldom necessary. A rule and a watch are sometimes useful for a rough comparison of rates of penetration.

The technique, though apparently simple, is not easy and requires a good deal of practice on each of the different types of material. The difficulty lies in the fact that with most kinds of wood under nearly all conditions, not only a puncture or slit but also any very slight localized pressure on the wood surface is sufficient to disrupt the water columns or to damage the superficial tracts enough to allow air to enter and give the 'streak test'. It is therefore very difficult to remove a patch of bark without exerting too much pressure, by leverage, &c., on the area of the wood to be bared and therefore without causing streaks to appear in the apparently intact wood. Until the necessary skill has been acquired the streak test is always positive. The removal of the bark without causing streaks to appear can be accomplished, however, and once the technique has been acquired, since it can easily be repeatedly checked, one need have no hesitation in declaring that the streaks observed are naturally present in some cases.

The best procedure for larger branches (e.g. above 5 cm. diam.) appears to be to cut first of all two longitudinal incisions, about 4 cm. long and 3 cm. apart, side by side and penetrating to the full depth of the bark down to the wood. The depth of these is noted by inserting the tip of the scalpel, and two transverse incisions are then made, but not quite as deeply so as to enclose a rectangle with the others. Most of the bark in this rectangle is then removed by tangential insertions of a scalpel—care being taken that nowhere is the whole depth penetrated—until only a thin (e.g. 1 mm.) layer of phloem remains. This is sufficient to protect the wood up to this point unless very hard or very local pressure be applied. Where any leverage of the bark with a scalpel is applied it is very important that the point of the scalpel acting as the fulcrum be not allowed to rest upon the bark of the area to be exposed but only on contiguous areas, *not* above or below it. The bark at the sides of the area is then bevelled off to allow of easier access to the bared area. The remaining layer of phloem is then raised slightly by leverage with the tip of the scalpel at one side, and, while held away from the wood, is cut across slowly half way up by means either of a second scalpel or a fine pair of scissors. When the cut is once started the next part to be cut may usually be held away from the wood surface by pulling on one of the already cut edges with a pair of forceps instead of

continuing the leverage with the scalpel. Throughout the operation the instruments used must not touch the surface of the wood. When the transverse cut is complete the remaining phloem layer is bent back in two flaps—one upwards and one downwards—and detached with a scalpel or scissors with the same precaution so as not to touch the wood. The surface exposed is then immediately examined for streaks and general degree of translucency, and the plain puncture test, the ink or eosin test, and the water test are applied as already described. The transverse cut across the phloem is usually made from right to left and all levering done by using the bark on the right-hand side as the fulcrum. If, for repeated tests, a larger area than that originally exposed be necessary it is a comparatively simple matter to extend the area to the left by similar means, and there is no risk of damage by the earlier manipulations to the wood then exposed. For smaller branches the same principles are applied on a smaller scale.

Materials Used and Conditions of Experiments.

Experiments have been performed on the lines described above upon the following plants: *Acer pseudoplatanus*, *Aesculus hippocastana*, *Alnus glutinosa*, *Corylus avellana*, *Crataegus oxyacantha*, *Fraxinus excelsior*, *Hedera helix*, *Ligustrum* sp., *Sambucus nigra*, *Tilia europaea*, *Ulmus campestris*, and *Viburnum lantana*. Of these the most definite and diagrammatic results, especially with the puncture test, upon which everything else turns, were given by *Sambucus nigra*, *Hedera helix*, and *Fraxinus excelsior*, though *Viburnum lantana* and *Crataegus oxyacantha* also afforded definite results comparatively easily. *Sambucus nigra* is one of the most 'diagrammatic' and is to be recommended for practising the technique in the early stages and for observing the appearance of streaks, since the wood is very easily injured and very readily shows up faulty manipulation. Trunks and branches of different sizes were used in most cases, varying possibly from 1 cm. to 20 cm. in diameter. The results with branches of different sizes are generally exactly the same. The only exceptions are indicated in the tables. The larger sizes are naturally more difficult to work with on account of the toughness and thickness of the bark, but with practice are all amenable to treatment.

All the observations made so far and recorded here have been made at a height of 1–2 metres from the ground, and the experiments have been carried out mainly during the few weeks preceding and the few weeks following the opening of the leaf-buds in spring (March, April, and May). Further experiments are in progress, however, dealing with various heights from the ground up to 15 metres and covering the whole of the yearly cycle. In the earliest experiments the wood was bared under paraffin, but this was later found to be unnecessary.

The experiments were performed partly at Eynsford, Kent, and partly in the grounds of Queen Mary College.

EXPERIMENTAL RESULTS.

The general results obtained are summarized in Tables I-XII. All the different types of experiment have been repeated at least 25 times on each of the species. Each line in the tables therefore represents the result of some 25-50 experiments. The results of the individual sets of experiments were remarkably consistent, being completely uniform in most cases and never showing more than 4 per cent. of discordant readings in any series.

TABLE I.

Experiments in Early Spring shortly before the Opening of the Leaf-buds.

Time : 9.0-11.30 a.m.¹ Conditions : Damp after rain at night.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Crataegus oxyacantha</i>	—	+	+	—	No air present
<i>Fraxinus excelsior</i>	—	+	+	—	No air present
<i>Hedera helix</i>	—	+	+	—	No air present
<i>Sambucus nigra</i>	—	+	+	—	No air present
<i>Ulmus campestris</i>	—	+	+	—	No air present

TABLE II.

Experiments in Early Spring (as in Table I), but on Hot Sunny Afternoons after Two Days' Rain.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Alnus glutinosa</i>	—	+	+	—	No air
<i>Crataegus oxyacantha</i>	—	+	+	—	No air
<i>Fraxinus excelsior</i>	+	+	++	—	Little air
<i>Hedera helix</i>	—	+	+	—	No air
<i>Sambucus nigra</i>	—	+	+	—	No air
<i>Ulmus campestris</i>	—	+	+	—	No air

TABLE III.

Later Spring, shortly after the Opening of the Leaf-buds.

Time : 10.0 a.m.-4.0 p.m. Conditions : Warm sun after 1-2 days without rain.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Acer pseudoplatanus</i>	++	+	+++	+	Air present
<i>Aesculus hippocastana</i>	++	+	++	+	Air present
<i>Alnus glutinosa</i>	++	+	++	+	Air present
<i>Corylus avellana</i>	++	+	++	+	Air present
<i>Fraxinus excelsior</i>	+++	+	+++	+	Air present
<i>Sambucus nigra</i>	++	+	+++	+	Air present
<i>Tilia europaea</i>	++	+	+++	+	Air present

¹ G.M.T.

TABLE IV.

*Late Spring after Opening of the Leaf-buds (as in Table III), but at 4 a.m.
on Very Wet Mornings after Much Rain.*

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Alnus glutinosa</i>	—	+	+	—	No air present
<i>Corylus avellana</i>	—	+	+	—	No air present
<i>Fraxinus excelsior</i>	+	+	+	+	Little air present
<i>Sambucus nigra</i>	—	+	+	—	No air present

TABLE V.

Late Spring (May): Damp Mornings after Little Rain.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Acer pseudoplatanus</i>	+	+	+	+	$\frac{1}{4}$ full of air
<i>Ligustrum</i> sp.	+	+	+	+	$\frac{1}{4}$ full of air
<i>Sambucus nigra</i>	—	+	+	—	No air

TABLE VI.

Late Spring (May): Hot Sunny Afternoons.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Acer pseudoplatanus</i>	++	+	++	+	$\frac{4}{5}$ full of air
<i>Ligustrum</i> sp.	++	+	++	+	$\frac{3}{5}$ full of air
<i>Sambucus nigra</i>	+	+	+	+	$\frac{1}{4}$ full of air

TABLE VII.

*Late Spring (May): during Heavy Rain after Much
Rain at Night.*

Material.	Streak. test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Acer pseudoplatanus</i>	+	+	+	+	Over $\frac{1}{2}$ full of air
<i>Ligustrum</i> sp.	+	+	+	+	Over $\frac{1}{2}$ full of air
<i>Sambucus nigra</i>					
Young green stems	—	+	+	—	No air present
Larger woody stems	+	+	+	+	Some air present

TABLE VIII.

*Experiments in June on Hot and Sunny Days during a Spell of
Fine Weather.*

Time: 10 a.m.—12 noon.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Acer pseudoplatanus</i>	++	+	+++	+	Much air present
<i>Fraxinus excelsior</i>	+++	+	+++	+	Much air present
<i>Ligustrum</i> sp.	+	+	+++	+	Air present
<i>Sambucus nigra</i>	+	+	+++	+	Air present

TABLE IX.

Experiments in June during Wet Weather after Several Days' Rain.

Time: 10 a.m.-12 noon.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Acer pseudoplatanus</i>	+	+	+	+	Little air present
<i>Fraxinus excelsior</i>	+	+	+	+	Little air present
<i>Ligustrum</i> sp.	-	+	+	-	No air present
<i>Sambucus nigra</i>	-	+	+	-	No air present

TABLE X.

Experiments in September.

Time: 10.0-11.30 a.m. Conditions: wet after rain.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Acer pseudoplatanus</i>					
Superficial wood	-	-	-	-	Not conducting
Deeper wood	-	-	+	-	No air
<i>Alnus glutinosa</i>					
Superficial wood	-	-	-	-	Very wet; no air
Deeper wood	-	-	+	-	Very wet; no air
<i>Corylus avellana</i>	-	-	- or very slow	-	No air; pressure almost = atmospheric (50 expts.)
<i>Fraxinus excelsior</i>					
Superficial wood	-	- or very slow	+	-	No air; very slightly reduced pressure
Deeper wood	-	+	+	-	No air, but lower pressure than in outer layers
<i>Viburnum lantana</i>	-	++	++	-	No air

TABLE XI.

As in Table X, but Less Wet and during Sunshine.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Alnus</i> , deeper wood	+++	-	+	+	$\frac{3}{4}$ full of air; slightly reduced pressure (20 expts.)
<i>Alnus</i> , superficial wood	-	+	+	-	No air

TABLE XII.
Experiments in October.

Time: 10.0–11.30 a.m. Conditions: damp after rain.

Material.	Streak test.	Puncture test.	Ink and eosin.	Water test.	Conclusion.
<i>Acer pseudoplatanus</i>	—	—	+	— (?)	Full of air (?)
<i>Aesculus hippocastana</i>	—	—	+	— (?)	Full of air (?)
<i>Alnus glutinosa</i>	—	—	+	— (?)	Full of air (?)
<i>Acer</i>	+ deeper wood	—	+	—	$\frac{1}{8}$ full of air
<i>Aesculus</i>					
<i>Alnus</i>					
<i>Fraxinus excelsior</i>	—	—	±	—	No air; atm. pressure
<i>Hedera helix</i>	—	++	++	—	No air
<i>Sambucus nigra</i>	—	++	++	—	No air

CONCLUSIONS.

It will be observed from Table I that in early spring, before the expansion of the leaves, no air was ever detected in the superficial conducting tracts during damp conditions after rain. A tension always existed in the tracts, as shown by the invariable penetration of ink and eosin in the ink and eosin tests, and a light streak was always produced on puncturing with a dry needle, showing the entry of air. Water could never be seen entering in the water test and streaks were never naturally present before puncturing. Air was definitely absent.

During hot, sunny conditions (Table II) the same results were obtained, except in the case of *Fraxinus*; this exceptional result is evidently related to the fact that at the time of the experiments the inflorescences had already opened, so that a greater tension would be set up by transpiration in this than in the other cases. It is correlated also with a more rapid intake of eosin and ink. *Corylus* and larger branches of *Alnus* and *Fraxinus* (e.g. 20 cm. diam.), at this time gave only inconclusive results.

Experiments performed a few weeks later, after the opening of the buds, show totally different results (Table III). Here, throughout the hotter parts of the day during fine weather, air is found always to be present in the tracts of all the species investigated. Absorption of eosin and ink is vigorous, and streaks of air-containing tracts are always visible, the streaks rapidly filling with water on application of the water test. As far as could be judged, from 20 to 50 per cent. of the tracts contain air in these conditions, the largest numbers being found in the Ash. Here, however, larger trunks (up to 20 cm. diam.) than in the other species were investigated, and it generally appeared in this case that more tracts were occupied by air in the larger stems than in the smaller.

Experiments upon the same species at the same time of year, but during wet weather, and carried out in the early morning (4 a.m. G.M.T.), show, however, that in these conditions and at this time of day air is not

usually present (Table IV). Absorption of ink, dye, or air was relatively weak in all such cases, showing that the pressure in the tracts was only slightly below that of the atmosphere. Absorption was least weak in the case of the Ash (*Fraxinus*). Only in the case of the Ash, too, was any air found to be present, and here it was only present as far as could be observed in from 10–20 per cent. of the superficial tracts. Thus air present during the daytime on a warm day disappears again in most cases during the night.

Another series of experiments carried out at this time of year upon *Acer*, *Ligustrum*, and *Sambucus* under various conditions is recorded in Tables V–VII. The results for *Sambucus* are in good agreement with those of the other series (Tables II–IV), and show that the air which is sometimes present disappears at least from the younger stems during the wetter conditions. In the other two forms (*Acer* and *Ligustrum*) the air appears to be more persistent, as it never entirely disappeared at this time during the conditions investigated. The estimates of the percentages of the tracts containing air can of course be regarded as only approximate. In some cases (Elder, &c.) they can be obtained by comparing the relative widths of the streaks and the darker areas of the wood. In other cases, as in the Ash, where the streaks represent individual tracts, they are obtained by making a small transverse slit in the bared wood surface. A number of counts is then made of the new streaks appearing between each pair of existing streaks. This gives the mean number of water-containing tracts to one air-containing tract in the intact plant. Thus, if on the average three new streaks appear on slitting between each pair of adjacent streaks originally present, the wood is described as one quarter full of air and so on.

Experiments during hot, sunny weather after a dry spell in June (Table VIII) show that in these conditions there is considerable tension in the tracts, and that air is always present during the middle of the day. In the Ash it appeared that about two-thirds of the tracts were put out of action by air. After several days' rain, however, as shown in Table IX, the air disappeared in two out of the four species investigated, and in the others (*Acer* and *Fraxinus*) remained only in much smaller amounts. *Alnus*, *Tilia*, and *Aesculus* unfortunately only gave inconclusive results in June, as the superficial wood at this time of year appears to be of such a nature that the entry of air does not cause a visible streak: neither can the entry of water be followed, although eosin and ink are easily seen to penetrate. It is possible that when this happens the tracts are full of water under very slightly reduced pressure. Ink, eosin, and water might then all penetrate slowly (the water, however, not being seen, as the tracts are already full), and air might not enter on account of a difference in pressure insufficient to overcome the surface-tension effects at the puncture.

The results of the remaining series recorded in Tables X–XII are not quite as consistent as those of the earlier experiments, nor quite as conclusive. The superficial wood at this time of year evidently has in most cases little to do with conduction, and interferes with the observation of the deeper wood. When the superficial wood is removed under water, lighter and darker streaks may be seen very often in the conducting wood, of which only the darker are capable of absorbing dye or ink. The lighter areas are fibres and are to be disregarded. Hence one can only conclude that the streak test is positive and that air is present if, by the methods given on p. 369, the streaks observed can be shown to be true tracheal streaks and not fibre-streaks. Allowing for this, it appears reasonably certain that air is not normally present in most cases during wet conditions in early autumn (September), (Table X), and that the pressure in the tracts at these times is but slightly lower than that of the atmosphere. In the case of *Alnus* during less wet conditions with sun, however (see Table XI), about three-quarters of the tracts of the deeper wood appeared to be occupied by air, although the whole of the wood was very wet and the superficial layers definitely contained no air. It is not of course impossible that the air had been admitted during the removal of the outer wood, but the results are in agreement with those obtained in later autumn (Table XII), from which it appears that at this time much of the outer wood is occupied by air in *Alnus*, *Acer*, and *Aesculus*. The wood, moreover, was so wet, and the pressure in the tracts evidently so slightly reduced, that it is unlikely that air would enter to this extent as a result of faulty technique.

The October experiments recorded in Table XII show definitely that during damp conditions after rain air is not present at this time of year in *Hedera helix*, *Sambucus*, or *Fraxinus*. In the cases of *Alnus*, *Acer*, and *Aesculus*, however, it seems that the outer wood is completely occupied by air, as the streak test is always negative, and ink and eosin penetrate rapidly, but only for a short distance. In this case the water test should be positive, but appears negative; this may be due to the difficulty of seeing the water column when it has entered. In woods of these types it seems more likely that a penetrating water column would be overlooked in wood containing only air than that a penetrating air column would be overlooked in wood that was completely saturated with water. It is also likely that if air and water were both present the differences would be observable as in the other cases. Elder wood at this time is normally full of water, but if the bared surface be scraped all the superficial tracts become filled with air, and the appearance then presented is very similar to that of the *Acer*, *Alnus*, and *Aesculus* woods at this time, all of which suggests that the wood in these cases contains air. The superficial wood is almost entirely non-conducting, taking up stain and ink only in places, and consisting principally of fibres filled with air. In the deeper wood (i.e. the outermost layers of conducting

wood), as shown by removing the superficial wood under water, it appears that about one-third of the tracts contain air, though the results are not quite as convincing as those obtained at other times of the year. Although the determination of the presence or absence of air at this time of year is not the most important issue, it is hoped that in the course of further experiments further data may be obtained upon this and other points.

The most important general conclusion is that at least during the early parts of the year air is sometimes present and sometimes not. The amount of air or the presence or absence of air in the tracts depends upon the external conditions, and therefore there must be times at which the physical conditions in the xylem are such that air-bubbles once formed can again disappear. A more thorough investigation on the lines indicated of the times and conditions during which air appears and disappears should help considerably in the formulation of a hypothesis on the mechanism by which tracheal air is forced again into solution. Such an investigation is now being undertaken.

SUMMARY.

Simple experiments are described for the detection of the presence of air in the superficial conducting tracts of trees. If due precautions be taken, tracheae containing air can be seen as lighter-coloured streaks on the surface of the wood after the careful removal of the bark. Similar streaks are produced by puncturing the surface of the stripped wood, and so admitting air to the conducting tracts. Very suitable materials are *Sambucus nigra* and *Syringa*, which give most diagrammatic results.

By the use of this method it is shown that air is sometimes present and sometimes absent from the conducting tracts, the presence or absence largely depending on external conditions. A mechanism must therefore exist for causing bubbles once formed to pass back into solution.

An Improved Electrical Conductivity Method for the Estimation of Carbon Dioxide and other Reactive Gases.

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With eight Figures in the Text.

THE measurement of carbon dioxide is one of the most important physiological estimations and many methods have been perfected for this purpose. In many cases the estimation involves minute quantities and, furthermore, not only is it desirable to measure total quantities but also rates of production which may be both variable and slow. Continuous methods, capable of high sensitivity, are therefore very desirable.

The manometric methods developed by Barcroft have been applied to Plant Physiology by Warburg and others and have recently been described in detail by Dixon (8). The katharometer has been developed for use in physiological investigations and high sensitivity makes it eminently suitable for the estimation of small quantities. A long acquaintance with it is, however, necessary before reliable results can be obtained.

The electrical conductivity method has also been adapted for continuous measurement of small quantities of carbon dioxide. This paper deals with a modification of this method for estimation by absorption in baryta of any quantity of carbon dioxide down to very low levels; the method is of high precision, and capable of following changes continuously within short periods of time. The electrical conductivity method was apparently first applied to the estimation of carbon dioxide in 1919 by Cain and Maxwell (4) for the estimation of carbon in steel. They were content with an apparatus of low sensitivity and were concerned only with the total volume of carbon dioxide absorbed.

The first application of the method to biological problems was by Spoehr and McGee (21) in 1923, who measured respiration of whole plants or several leaves over long periods of time. The concentration of baryta was reduced from 0.12 N. to 0.05 N., which latter they considered to be the

weakest solution capable of completely absorbing all the carbon dioxide from a rapid air stream. The presence of the barium carbonate precipitate increased the difficulties of the method owing to deposition on the electrodes. These investigators, for this reason, abandoned attempts to make the method one of continuous reading. After absorption the baryta solutions were placed in stoppered bottles and, after allowing the precipitate to settle, the conductivity was determined by dipping electrodes. A later communication (22) in 1924, outlines improvements in the method for increasing the sensitivity by reducing the volume of the baryta from 125 to 75 c.c. The calibration of the cell was carried out with atmospheric air as a dilute constant mixture of carbon dioxide, a procedure which they justified by reference to the work of Benedict (2).

An extremely sensitive method capable of detecting 10^{-7} gm. of carbon dioxide was described by W. O. Fenn (9) in 1926 for use in the estimation of carbon dioxide output by stimulated nerves. High sensitivity was obtained by using a small volume of very dilute baryta (7 c.c. of 0.00475 M.). Owing to the small quantities no difficulties of changing cell constant, due to carbonate precipitate, seem to have arisen. The rate of absorption in the dilute solution was rather slow, and to obviate this difficulty the air was continuously circulated over the nerve tissue and through the conductivity cell. In a later paper (10), in 1928, further improvements in the method were described, and the sensitivity was raised to 0.8×10^{-7} gm. of CO_2 .

Continuous readings of carbon dioxide production over short periods of time were made by Bayliss (1) in 1927; it would appear that Bayliss was unaware of any previous application of the conductivity method. Absorption was carried out in soda solution and the conductivity variations were due to the change in migration velocity by replacing hydroxyl ion by carbonate ion. This method is less sensitive than absorption in baryta, but has the advantage that no precipitate is present. Further, the initial concentration of sodium hydroxide can be made considerably greater than that of baryta, so that for a given cell volume larger quantities of carbon dioxide can be measured.

In 1927 an improvement on the method of Spoehr and McGee was described by Raymond and Winegarden (19); these investigators carried out careful measurements on the conductivity of baryta solutions at different concentrations and temperatures to supplement the scanty information on this subject. Determinations were made with an accuracy of 6×10^{-7} gm. of carbon dioxide per c.c. of absorbing solution.

The Theory of the Conductivity Method.

In the following discussion certain simplifying assumptions are made

(a) that all salts present are completely ionized in the sense of the term as used by Arrhenius,

(b) that ionic mobilities are unaffected by changes in concentration,
(c) that the law of independent migration of the ions holds strictly true,
and that no solute is adsorbed on the precipitate.

Let $x_1 \dots x_2 \dots x_3$ be the numbers of ions of different species per c.c.

Let $r_1 \dots r_2 \dots r_3$ be the numbers of ions of different species produced by the addition of one gram of the reacting gas. Note that when ions are removed by the gas the sign of the corresponding r will be negative. Let q be the weight of the reacting gas introduced into the system of volume v . Then the change in number of ions present (dx) is equal to the change in the amount of gas added (dq) multiplied by the number of ions produced per c.c., per gram of gas, i.e.:

$$dx = \frac{dq \cdot r}{v} \dots (1).$$

Now if $k_1 \dots k_2 \dots k_3$ be the specific conductivities of the various ions, the total conductivity will be:

$$c = \Sigma kx \dots (2).$$

and

$$dc = \Sigma kdx \dots (3).$$

therefore

$$dc = \frac{dq}{v} \Sigma kr \dots (4).$$

so that $\frac{dc}{dq}$ is a constant and equal to $\frac{\Sigma kr}{v}$. From the above relation:

$$dq = \frac{v \cdot dc}{\Sigma kr} \dots (5).$$

If changes in conductivity are measured by means of a Wheatstone's bridge, the sensitivity of the bridge (s) is determined by the relation $s = \frac{dl}{l}$, where dl is the smallest movement of the contact on the bridge wire which can be detected, and l is the length of the wire. It can be shown that $s = \frac{dc}{c}$ so that:

$$dq = \frac{v \cdot s \cdot c}{\Sigma kr} \dots (6).$$

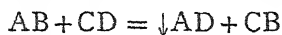
and

$$dq = \frac{v \cdot s \cdot \Sigma kx}{\Sigma kr} \dots (7).$$

From the above relation it is clear that, theoretically, there is no limit in smallness of the amount of gas measurable: dq may be decreased either by decreasing the volume of the absorbant (v) or decreasing the concentration of the absorbant (Σkx). The smallest measurable quantity may be decreased also by increasing the sensitivity of the bridge i.e., decreasing the magnitude of s by using a longer wire or extension coils. In practice, however, there is a limit beyond which the volume cannot be reduced or

the concentration decreased, so that the sensitivity of this method is largely determined by the efficiency of the absorbing cell.

The theoretical discussion of the method includes cases in which no precipitate is formed, such as absorption of carbon dioxide in soda (Bayliss (1)) or cases in which a precipitate removes the gas, e.g., in absorption of carbon dioxide in baryta. Consider the reaction:



where AB is the substance in solution in the cell, CD the reacting gas, or its compound with water, and AD is removed by precipitation. Suppose the values of the specific conductivities of the A, B, C, D, ions are k_a, k_b, k_c, k_d respectively. As the B ions remain in solution they do not affect the conductivity, and the ions of AD are removed by precipitation. Hence the number of ions produced by the gas (in the nomenclature used above) is given by $r_c - r_a$. Hence:

$$\frac{dc}{dq} = \frac{1}{v} (k_c r_c - k_a r_a).$$

The conductivity will therefore either increase or decrease as $k_c r_c$ is greater or less than $k_a r_a$. A case in which the conductivity increases is exemplified by the estimation of hydrogen sulphide by absorption in silver nitrate:



As both silver and hydrogen are monovalent $r_{Ag} = r_H$ and the values of k are proportional to the ionic mobilities (u), hence:

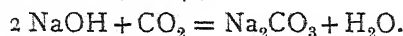
$$\frac{k_H}{k_{Ag}} = \frac{u_H}{u_{Ag}} = \frac{330}{56}$$

therefore

$$\frac{dc}{dq} = + \frac{1}{v} \left(\frac{274}{330} k_H r_H \right).$$

The positive sign shows the increase in conductivity and the value $\frac{274}{330}$ is proportional to the magnitude of this increase.

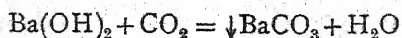
A decrease in conductivity is obtained in the absorption of carbon dioxide in soda as used by Bayliss (1):



In this case no precipitate is formed, but the water produced is practically un-ionized. In this case $r_{OH} = 2 r_{CO_3}$ and $\frac{u_{OH}}{u_{CO_3}} = \frac{180}{40}$ so that:

$$\frac{dc}{dq} = - \frac{1}{v} \left(\frac{8}{9} k_{OH} r_{OH} \right).$$

Absorption in baryta presents an interesting case as neither of the products are ionized:



in this case

$$\frac{dc}{dq} = -\frac{1}{v} \frac{415}{360} k_{OH} r_{OH}$$

This result can be compared with that obtained with soda, in which :

$$\frac{dc}{dq} = -\frac{1}{v} \left(\frac{320}{360} k_{OH} r_{OH} \right).$$

The baryta method, therefore, is more sensitive than the soda method in the proportion of $\frac{415}{320} = 1.3$. Arising from the discussion it is clear that theoretically, with complete ionization, the relation between change in conductivity and mass of gas added should be linear.

Description of Apparatus.

Electrical equipment.

An alternating current Wheatstone's bridge was used to measure the resistance of the cell, the A.C. supply at 960 cycles per second being taken from a thermionic valve oscillator, as originally suggested by Taylor and Acree (25). Details of circuits may be obtained from the text-books of Davies (5) and Britton (3). The bridge was a straight wire 100 cm. long with no extension coils, giving a value of $s = 0.04$ per cent.

As is essential with alternating current measurements, a variable-capacity air condenser was used in parallel with the resistance box (Fig. 1) to balance the capacity of the cell, it being impossible otherwise to obtain a sharp end-point. Headphones were used to detect the current when the bridge was out of balance, as recommended by Washburn and Parker (26), the sensitivity being greatly increased by using a valve amplifier (Fig. 1). It was found very convenient to use a new type of indirectly heated thermionic valve (Ostar-Ganz) in the oscillator and amplifier, taking 210 volts on the heater, which can be run directly off the mains, thus obviating the inconvenience of accumulators and batteries. The conductivity cell was immersed in a constant temperature bath kept at 25°C . with a temperature variation of about 0.01°C . The water in the thermostat was earthed.

In determining the balance point of the bridge it is often convenient to be able to detect whether the contact on the wire is above or below the null point. With the alternating current bridge as described above, the detector is unable to distinguish the direction of flow of the alternating current. The following device, however, for which the author is indebted to Mr. Baggally, overcomes this difficulty. Advantage is taken of the change in phase which occurs as the contact passes from one side of the null point to the other. A small current of constant phase, sufficient to produce a deflection in the galvanometer, is superimposed on the current from the bridge through the rectifier. If the bridge is out of balance and

the superimposed alternating current is flowing in phase with the current from the bridge the galvanometer deflection will be increased. If the contact is now moved to the other side of the null point the two currents will be out of phase and a decrease in the galvanometer deflection will occur.

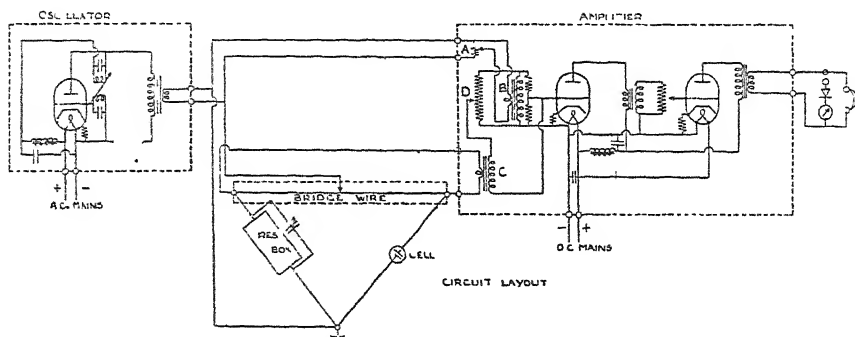


FIG. 1. Circuit layout. For explanation of lettering see text. In the oscillator the condenser has been omitted between the transformer and the negative of mains.

The constant phase current is introduced into the amplifying circuit through a transformer (B, Fig. 1) similar to that (C) feeding the amplifier from the detector circuit. To prevent a transfer of energy between the transformers they are arranged as the diagonals of a balanced bridge. The magnitude of the superimposed alternating current is controlled by the potentiometer, A (Fig. 1). The potentiometer D is used to balance the transformer bridge.

The conductivity cell.

The conductivity cell used in this work was specially designed to meet the following requirements: (i) efficient absorption, (ii) small volume, (iii) removal of precipitated barium carbonate, (iv) avoidance of 'skin effects' at the liquid surface.

The general design of the conductivity cell is shown in Fig. 2. It consists of a spiral of tubing (A), a filter chamber (B), and an electrode chamber (C). The stream of air containing the reacting gas enters the apparatus at (D) and is injected into the spiral through the capillary (E), and there broken up into bubbles. As the absorbing solution stands at a higher level ($a \dots a$, Fig. 2), the bubbles lift the solution up the spiral into the filter chamber (B). The air does not pass in the reverse direction through the electrode chamber because of the assistance due to the closely fitting filtering thimble. The reacting gas is absorbed in the spiral during its passage and the precipitated material either remains as a thin layer on the walls of the spiral or is carried over. Considerable difficulty was experienced in finding a suitable filter which would remove the barium carbonate from suspension and yet allow the solution to filter rapidly.

Eventually it was found that a 16 mm. Soxhlet extraction thimble was perfectly satisfactory. The air stream finally passes out at (F) and the solution passes through the thimble into the electrode chamber.

Special reference should be made to the method of inserting the electrodes suggested by Dr. J. I. Armstrong, of this Institute. The electrodes consist of pieces of stout platinum foil, approximately 7 sq. mm. in area welded into holes in the walls of the electrode chamber. Being flush with the wall there is no dead space around them, and as they are rigidly supported, no relative movement is possible. In practice, the platinum foil is first fused into the end of a glass tube (which is later filled with mercury, thus making electrical contact (Fig. 2); these tubes are then fused into the wall of the electrode chamber. This procedure decreases the amount of 'creeping' of the glass over the inner face of the electrodes. The surfaces of the electrodes were well platinized to reduce polarization. No adsorption of baryta was detected.

The air stream keeps the absorbing solution circulating over the electrodes, and continuous readings can be taken. As the free surface of the liquid in the filter chamber is considerably distant from the electrode surfaces, all 'skin-effects' are successfully eliminated.

Calibration of the cell.

The method adopted to determine the cell volume was to weigh before and after filling with the absorbing solution, and it was found worth while to construct a special pipette to deliver approximately the correct volume. This volume should be such that the end of the tube, G (Fig. 2), reaches just below the level of the solution in the thimble when the air stream is passing, the bubbles then produced mix the contents of the filter chamber.

Sixteen experimental cells of various sizes were constructed for use in the estimation of different amounts of carbon dioxide. The bore of the spiral, however, imposes a limit, as bubbles fail to pass up in very fine tubes. A cell of volume 250 c.m. has been successfully used, and in this

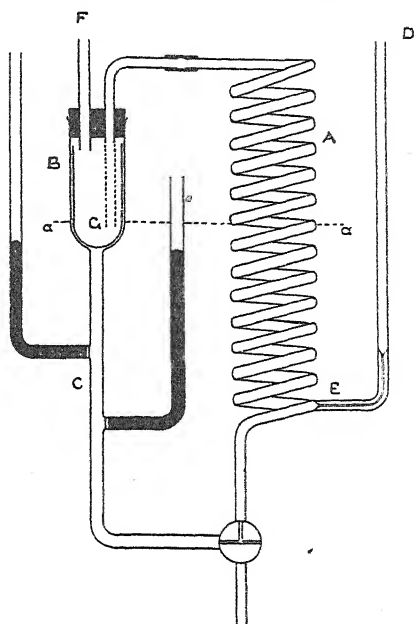


FIG. 2. Conductivity cell. For explanation see text.

case complete absorption was obtained with a straight tube in place of the spiral.

The cells were calibrated by drawing moist air from a cylinder of compressed air through the cell. The volume of air passed and the change

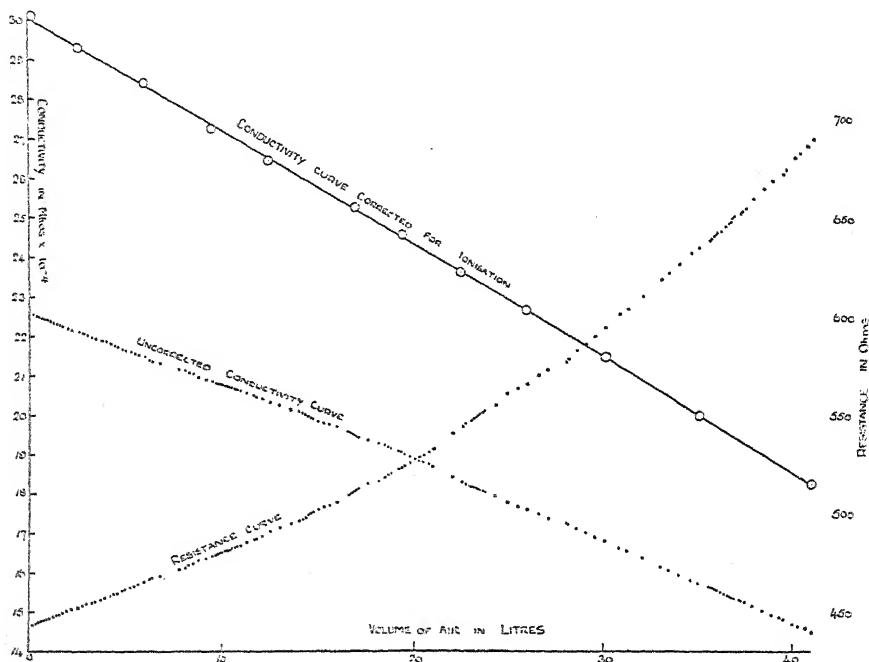


FIG. 3. Calibration of fifth experimental absorber. Curves connecting volume of air with resistance, conductivity and corrected conductivity of cell.

of resistance was measured. The air used contained 5.59 mg. of carbon dioxide in 10 litres. Fig. 3 presents graphically the relation between the volume (litres) of gas mixture passed and the resistance (ohms) of the cell; from the resistance the conductivity was calculated. Values for conductivity and resistance are shown, each point being the experimental values of a single determination.

The conductivity curve, although approaching linearity, is slightly concave to the abscissa. As it was suspected that this curvature was due to change of ionization of the baryta with increasing dilution of the solution, the figures were corrected for ionization change. The data for ionization corrections were obtained from Landolt Bernstein, Noyes (16), Raymond and Winegarden (19), and the International Critical Tables. The corrected data are shown as a separate curve in the diagram, and it is seen that, in accordance with theory, the relation between conductivity and mass of gas absorbed is strictly linear. The higher level of this curve is

due to the fact that calculations were all made for complete ionization. Table I shows a few of the actual readings obtained.

TABLE I.
Fifth experimental absorber.

Volume of gas mixture (litres).	Resistance (ohms).	Conductivity (mhos $\times 10^{-6}$).
9.8	480.1	2083
9.9	480.5	2081
10.0	480.8	2080
10.2	481.5	2077
10.3	481.9	2075
10.4	482.3	2073
10.5	482.6	2072
10.7	483.4	2069
10.8	483.8	2067
11.0	484.2	2065

In Figs. 4 and 5 are shown the relation between conductivity and volume of air passed in two other experimental cells, in each case with the weights of baryta shown beside the curves.

The increase in sensitivity of the apparatus as the cell size is diminished is very evident, the total quantity of carbon dioxide estimated in Fig. 5 being about 1 mg.

The Sensitivity of the Cell.

If dq represents the addition to the cell of a small mass of gas, then as pointed out above:

$$dq = \frac{-v \cdot s \cdot \Sigma kx}{\Sigma kr}$$

therefore, with baryta:

$$dq = \frac{-v \cdot s \cdot (k_{OH} x_{OH} + k_{Ba} x_{Ba})}{-(k_{OH} r_{OH} + k_{Ba} r_{Ba})}$$

then as $x_{OH} = 2x_{Ba}$, and $r_{OH} = 2r_{Ba}$, we get:

$$dq = \frac{v \cdot s \cdot x_{OH}}{r_{OH}}$$

The above relation enables a calculation to be made of the smallest quantity of carbon dioxide which may be estimated with a given cell containing a given volume of absorbant. Using the data for the smallest cell so far successfully operated:

$$v = 0.25 \text{ c.c.}$$

$$s = 0.04 \text{ per cent.}$$

$$x_{OH} = 3 \times 10^{-19} \text{ for } 0.05 \text{ N solution.}$$

$$r_{OH} = 2.7 \times 10^{22}.$$

$$dq = \frac{0.25 \times 0.0004 \times 3 \times 10^{19}}{2.7 \times 10^{22}} = 1.1 \times 10^{-7} \text{ gm. CO}_2.$$

C c

The sensitivity may also be deduced from the calibration curves for different cells given in Figs. 3, 4, and 5, and are represented by the slopes of the lines of closest fit. The equations to these lines from the fifth, ninth, and

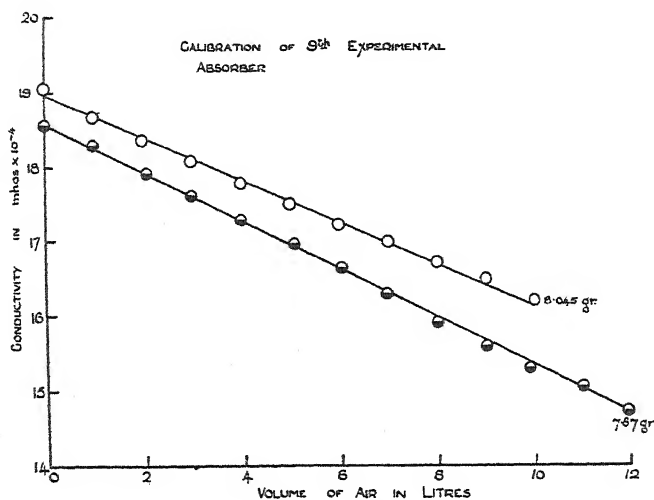


FIG. 4. Calibration of ninth experimental absorber.

tenth experimental absorbers are as follows, where y represents the conductivity in mhos $\times 10^{-7}$ and x is the number of litres of gas containing 0.559 mg. CO_2 per litre.

$$\begin{aligned} 5\text{th } y &= 30111 - 287.45 x \\ 9\text{th } y &= 18938.7 - 277.56 x \\ 10\text{th } y &= 10878.2 - 1011.1 x \end{aligned}$$

Since the sensitivity of the bridge is 0.04 per cent. the smallest reading of y possible can be calculated from the mean value of y' . The corresponding value of x can therefore be derived, and this represents the maximum sensitivity for the given concentration. The values obtained from the three calibrations are as follows, using 0.217 N baryta in each case:

5th	minimum quantity which can be estimated:— 18.3×10^{-6}				
9th	"	"	"	"	14.0 "
10th	"	"	"	"	2.24 "

From the relation given above the theoretical value has been calculated for the ninth absorber, the only case in which all the relevant data are to hand and the value obtained is 14.6×10^{-6} which agrees closely with the experimental value. The sensitivity could, of course, be increased by reducing the strength of the baryta solution, which would be feasible if the rate of flow of the gas were reduced in proportion.

Errors of Estimation.

The value of the sensitivity given above assumes that the experimental values lie strictly on the line of closest fit, The variance of the points about this line enables the real error of estimation to be calculated.

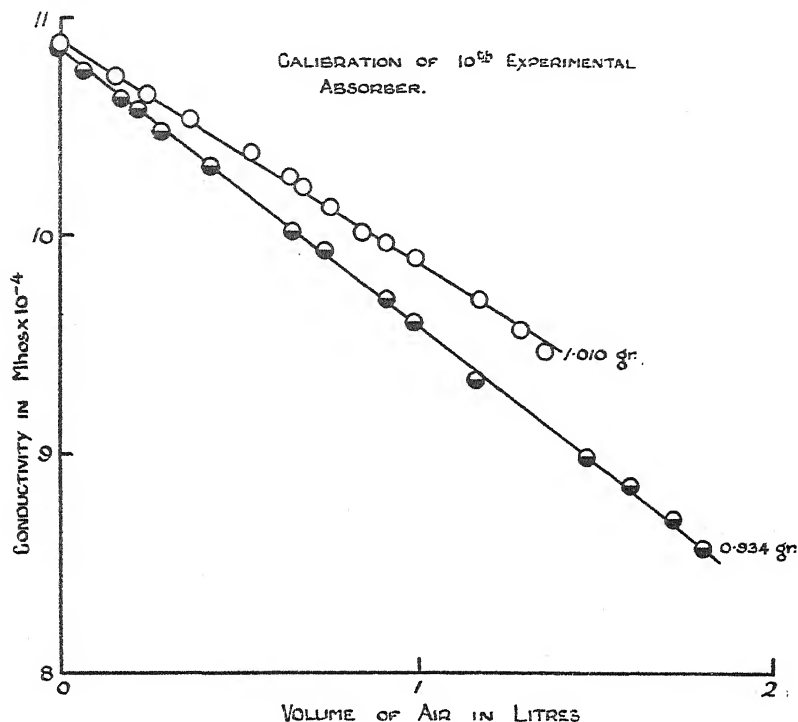


FIG. 5. Calibration of tenth experimental absorber.

The standard errors of the sensitivity of the three absorbers are as follows :

5th absorber (22 readings),	sensitivity	18.3×10^{-6} grammes CO_2	$\pm 1.36\%$	$\sigma = 6.08\%$
9th " (11 ")	"	14.0 "	$\pm 1.66\%$	$\sigma = 4.98\%$
10th " (14 ")	"	2.24 "	$\pm 1.48\%$	$\sigma = 5.13\%$

The figures show that (i) for a single determination the error of estimation is of the order of 5 per cent.; (ii) the error of estimation is independent of the size of the absorber used.

To test further whether consistent results can be obtained with a given cell, on passage of known quantities of carbon dioxide, the following method was used. A solution of sodium carbonate was made up, containing 0.9497 mg. carbon dioxide per cubic centimetre. 10 c.c. of this solution was decomposed in a Claisen flask with dilute sulphuric acid and the liberated carbon dioxide passed, with a stream of carbon dioxide free

air, into the conductivity cell, dissolved carbon dioxide being dispelled from the solution by gentle boiling. After correction the changes in conductivity were found to be in agreement within less than 1 per cent. The data are given below in Table II.

TABLE II.

Absorber number 11. Resistance of cell with 0.217 N. baryta, 433.0 ohms.

Experiment number.	Resistance (ohms).	Bridge reading (cm.).	Cell resistance (ohms).	Conductivity (mhos $\times 10^{-6}$).	Difference of conductivity.	Difference of conductivity corrected.	Divergence from mean.
1	500	49.85	508.1	1968			
	700	49.94	708.7	1411	557	654	4 (0.6 %)
2	470	49.61	482.2	2073			
	700	51.10	676.6	1478	595	663	5 (0.8 %)
3	500	49.78	509.5	1962			
	700	49.77	713.6	1401	561	660	2 (0.3 %)
4	450	49.10	471.0	2124			
	700	51.85	656.7	1522	602	655	3 (0.5 %)
					Mean 658		

As the same quantity of carbon dioxide was passed in every case, the baryta solutions were depleted to the same extent and, therefore, in making comparison, it was unnecessary to correct for the degree of ionization. A correction was also necessary for the amount of water in the cell before addition of baryta. Complete drying of the cell between experiments was tedious and unnecessary, and the practice adopted was to wash out and allow to drain and then to introduce a known weight of baryta. Carbon dioxide free air was then passed through the cell until no further change in resistance was noted, thus showing uniform mixing. The resistance of the originally completely dry cell was determined once for all, after addition of the standard baryta, and was found to be 433.0 ohms at 25° C. From this figure the values of the conductivity could be corrected in each case to the standard conditions of the dry cell. An illustrative example is given to make the method clear. Suppose the cell to have a 'dry' resistance of 400 ohms, i.e. 400 ohms when it contains only stock baryta; the conductivity will then be 2.5×10^{-3} mhos. Now suppose that sufficient carbon dioxide had been introduced to decrease the amount of baryta present by one quarter of its original amount, the conductivity will now fall to $\frac{3}{4} \times 2.5 = 1.875$, and $d\epsilon$ (the change in cond.) is 0.625. If now the cell had contained some distilled water remaining from the previous rinsing out, the resistance would be higher, say 500 ohms, the conductivity would then be $2.0 \text{ mhos} \times 10^{-3}$. Assuming, however, the same amount of carbon dioxide had been added, the conductivity would still have been reduced by $\frac{3}{4}$ and then becomes $\frac{3}{4} \times 2.0 = 1.5$ and $d\epsilon$ becomes 0.5. The 'wet' cell

value can be reduced to the 'dry' cell value by multiplying by the 'wet' resistance and dividing by the 'dry' resistance, i.e. $0.5 \times \frac{500}{400} = 0.625$. In this way the 'wet' cell values can all be reduced to the 'dry' cell values, as has been done in column 7 of Table II. The sensitivity is not affected by the amount of water present, as although the solution is weaker the volume is increased in the same proportion. In the same way two different cells can be compared, and such a comparison is shown below in Table III.

TABLE III.

A comparison of cell no. 9 ('dry' resistance, 488.9 ohms) with cell no 11 ('dry' resistance, 433.0 ohms).

Cell number.	Resistance (ohms).	Bridge reading (cm.).	Resistance of cell (ohms).	Conductivity (mhos $\times 10^{-6}$).	Conductivity (mhos $\times 10^{-6}$) corrected for ionization.	Difference corrected for ionization.	Difference corrected for ionization and 'dry' value.	Difference in corrected conductivities compared with cell no 11.
9	650	50.39	646.6	1546	2034	623	824	930
	900	50.20	502.2	1108	1411			
11	500	49.85	508.1	1968	2624	799	938	—
	700	49.94	708.7	1411	1825			

In this comparison the values of the conductivity have been divided by the degree of ionization to correct for the difference in the strengths of the solutions used. The 'dry' value was obtained as above, and the final comparison of cell No. 9 with cell No. 11 was obtained by multiplying 824 by $\frac{488.9}{433.0}$. The values for the same weight of carbon dioxide (9.5 mg.), as estimated by the two cells, differs by less than 1 per cent.

Experimental Results.

To show the behaviour of the instrument in practice some estimations are given of carbon dioxide production during respiration.

(1) Respiration of two pelargonium leaves.

Absorber No. 9. May 9, 1934. Temperature 21° C. Leaf areas (one side only) 75.22 and 74.39 sq. cm. The data obtained are presented in Table IV and shown graphically in Fig. 6. It will be seen that the amount of carbon dioxide produced per minute can be easily determined.

TABLE IV.

Time in minutes.	Bridge reading (cm.).	Resistance (ohms).	Conductivity (mhos $\times 10^{-7}$).	Carbon dioxide (mg.).
0	50.16	702.7	14321	—
6	49.81	712.4	14037	0.47
10	49.67	716.4	13958	0.60
15	49.45	722.5	13841	0.80
20	49.19	730.0	13698	1.03
28	48.83	740.3	13508	1.35
32	48.61	746.6	13394	1.54
37	48.47	750.5	13324	1.66
42	48.24	757.4	13203	1.86
48	47.99	765.1	13070	2.07
55	50.95	777.9	12855	2.43
61	50.69	785.8	12725	2.65
67	50.43	794.5	12586	2.88
72	50.18	802.5	12461	3.09

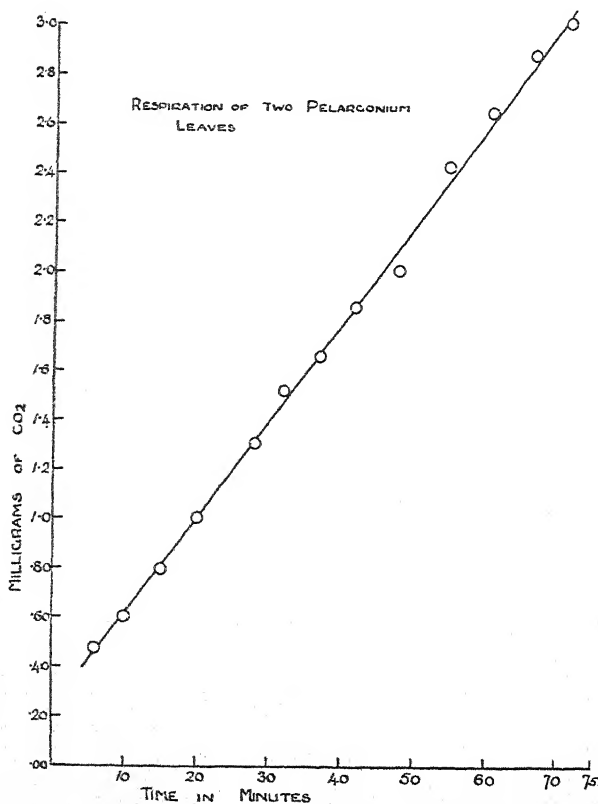


FIG. 6. Respiration of two pelargonium leaves.

(2) *Respiration of duckweed (Lemna minor).*

Absorber No. 10, containing about 1 c.c. of 0.2 N baryta, was used. Four samples of *L. minor*, each of approximately 100 fronds and grown under constant conditions of illumination, were obtained from Dr. E. Ashby of this Institute. The experiment was carried on for about thirty minutes in each case, and the results are expressed in terms of milligrams of carbon dioxide per 100 fronds. The data are presented in Table V and shown graphically in Fig. 7. Where estimations from more than one of the samples at a particular time were available they are given in the table. It is evident that low rates of carbon dioxide can be estimated over very short periods of time. Temperature 25° C.

TABLE V.

Time in minutes.	Carbon dioxide (mg.) per 100 fronds.		
1	0.005		
4	0.017	0.024	
9	0.043	0.043	
11	0.064		
15	0.073	0.078	0.078
16	0.081		
19	0.107		
20	0.106		
22	0.118	0.123	
26	0.148		
28	0.158		
32	0.180		

(3) *Other gases.*

The application of the method to other gases has been referred to in the general discussion. Some preliminary work was carried out on the estimation of hydrogen sulphide with silver nitrate. A dilute mixture of air and hydrogen sulphide was made up containing approximately 0.6 mg. H₂S per litre, and was then passed through a cell containing silver nitrate. The filtering device was found to work satisfactorily in this case also, as well as in the estimation of hydrochloric acid gas with silver nitrate. The results obtained with hydrogen sulphide are shown graphically in Fig. 8.

CONCLUSIONS.

The sensitivity of the method described compares favourably with other micro-methods. Thus the manometric method of Warburg is sensitive to 4×10^{-7} gm. of carbon dioxide. The katharometer, described by Slater (20), had a sensitivity of 1×10^{-6} gm. The improved katharometer of Stiles and Leach (23), as deduced from the calibration curve in their paper, seems to have a sensitivity of the order of 4×10^{-6} gm. CO₂. The indicator method of Osterhaut (17), Haas (12), Higgins and Marriott (13),

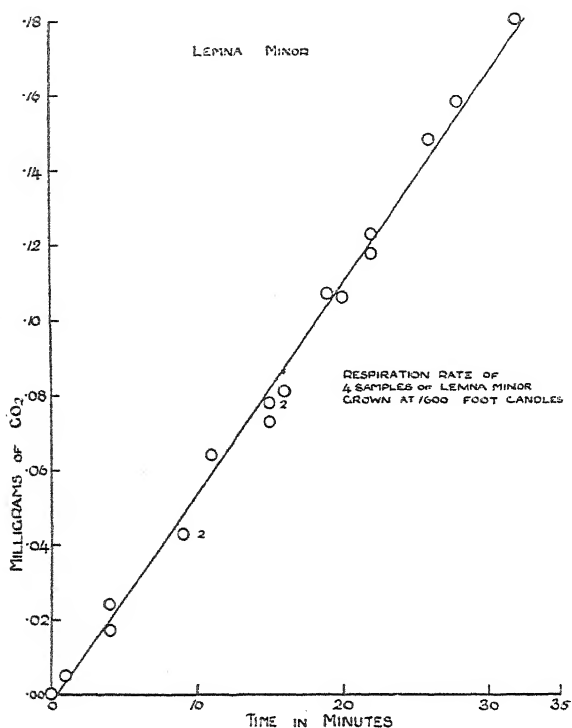


FIG. 7. Respiration rate of four samples of *Lemna minor* grown at 1,600 foot candles.

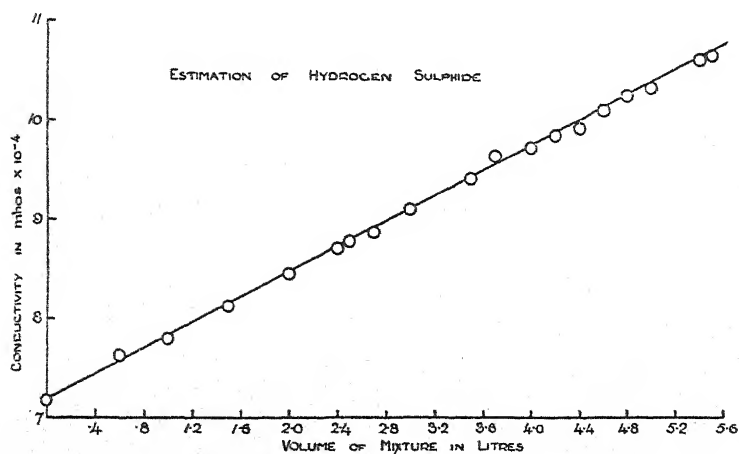


FIG. 8. Estimation of hydrogen sulphide.

Irwin (14), McClean and Denison (15), Parker (18), and others, can be made sensitive to about 10^{-6} gm. The micro-detection method of Tashiro is sensitive to 10^{-7} gm., but is tedious to operate. The values of the sensitivity give, however, no estimate of the accuracy with which determinations of this magnitude may be made.

The method here described has the following advantages:

(i) It is simple to use and is not very sensitive to temperature changes; (ii) it can be adjusted to any desired range of carbon dioxide, and the error of estimation is independent of the absolute quantity measured; (iii) the estimations may be made in a moving stream of air so that no accumulation of carbon dioxide occurs; (iv) readings may be taken over small intervals of time so that the instrument may be used for the determination of changes in rate of production with only a small time lag.

SUMMARY.

An improved conductivity method for the estimation of carbon dioxide by absorption in baryta is described.

An alternating current Wheatstone's bridge is used, the alternating current being derived from an oscillating thermionic valve and the sensitivity increased by the use of valve amplification.

A device is described to determine whether the position of the contact is above and below the 'null' point.

A conductivity cell of new design combining the advantages of high efficiency, complete removal of precipitate, and absence of 'skin effects', is described.

The theory of the instrument is discussed and calibration curves presented, showing that the relation between the change in conductivity and mass of gas added is strictly linear.

By using cells of different sizes, varying quantities of carbon dioxide can be measured, the accuracy being independent of the amount.

With cells so far used the highest sensitivity obtained was 1.1×10^{-7} gm. of carbon dioxide.

Continuous readings, over short intervals of time, can be taken, with a standard error, for a single determination, of 5 per cent.

The author wishes to record his thanks to Professor V. H. Blackman for much needed encouragement in the early stages of the investigation, to Dr. F. G. Gregory, under whose direction the work was carried out, and to Mr. Baggally and Mr. D. G. Reid for advice in overcoming technical difficulties of the electrical equipment.

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NOTES.

A 'BACK' FOR USE WHEN HONING MICROTOME KNIVES.—The usual 'back' used when honing plane-faced microtome knives consists of a split cylinder of brass, held in place by two screws. This device is open to the serious objection that both it and the cutting facets of the knife are ground down by the stone—the softer brass somewhat rapidly—so that the angle of the cutting facets is continually changing, and results sooner or later in the production of a long cutting wedge which, while satisfactory for relatively soft material, is not serviceable when the knife is used for cutting sections of hard substances like wood. In addition, the abraded brass particles from the 'back' tend rapidly to clog the pores of the hone, and consequently to decrease its cutting power.

These difficulties may be overcome by the use of a specially designed 'back' which is described in this note. The diagram (overleaf) is a scale drawing of the apparatus, which is used for Reichert knives 20 cm. long. A is a brass cylinder ($\frac{1}{2}$ in. by $\frac{5}{8}$ in. diameter), to the ends of which are fitted two roughly triangular brass plates B and B'. These plates are each attached by means of three taper-head screws, C. A brass knob, D, is fixed to the plate B, and serves for holding the knife steady upon the hone. A slot is cut out of the plate B, slightly larger than the back of the knife, allowing the knife to be slid into the 'back': the knife is held firmly in position by the screws E and E', which work through the brass sockets, F and F', soldered to the back of the cylinder A. G and G' are two silver-steel rollers, held in position by bearings passing through B and B'. In use the rollers rotate as the knife is pushed along the stone; wear upon them is thus reduced to a minimum; the cutting facets of the knife alone are ground, and as the rollers are not worn down, the angle of the cutting facets remains constant. Either end plate can be slipped off by removing its three screws, C, so that in the doubtful event of the rollers becoming worn, they are easily removed and replaced by new ones.

In practice, rollers of the size given in the drawing, or even thicker ones, have been found suitable for a knife of the size mentioned, when the knife is used for cutting sections of wood. Should it be desired to obtain longer cutting facets upon the knife, for use upon softer tissues, rollers of smaller diameter can be used— $\frac{1}{4}$ in. is recommended.

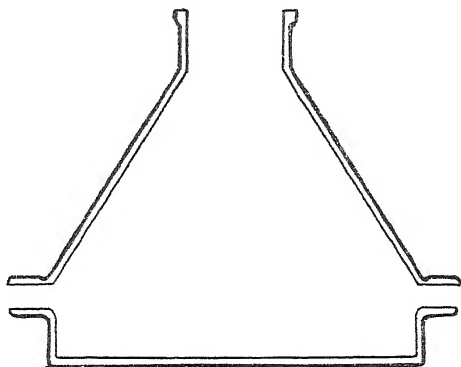
The back was made for me in the Department of Chemical Engineering at University College, London, by Mr. R. S. Potter, who, apart from one or two minor details, designed the apparatus. Mr. Potter originally suggested the use of two ball races in place of the rollers; while the amount of wear upon a ball race would have been even less than upon a roller, it was thought better to use rollers, as the width of the hone in use is much less than the length of the knife.

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November, 1934.

Notes.

A NEW TYPE OF CULTURE VESSEL.—Investigators who wish to preserve cultures of fungi and bacteria over an extended period for continuous study, or who desire to isolate pure cultures from mixed growths, are faced, under the conditions obtaining in most laboratories, with a number of obstacles. A Petri-dish culture has the double disadvantage that it tends to dry out relatively rapidly and that it is liable



to infection by air-borne spores. A flask culture, whilst avoiding these disadvantages, has other drawbacks, notably that introduced by the limitations imposed on manipulation by the narrow neck of the flask. It is a matter of common experience that the most desirable colony in a flask culture is so frequently in a position inaccessible to the platinum needle.

The apparatus described in this note has been designed to combine the accessibility and convenience of the Petri dish with the greater security against contamination and drying out provided by the flask. As will be seen from the accompanying diagrammatic sketch, it consists of two parts made of stout 'resistance' glass, the upper one shaped like a squat conical flask from which the bottom has been cut away and the lower one similar to half a Petri dish. Both upper and lower parts are provided with a flange, the two flanges being ground to give an accurate fit. In use the neck is plugged in the usual way and the medium poured into the lower part, the joint being vaselined; carbolized vaseline is used if this additional precaution is considered necessary. Cultures made in a vessel of the type described have remained healthy and uncontaminated over periods of many months when left on a laboratory bench, and a subculture has recently (October, 1934) been obtained from an inoculation made in May, 1933.

It will be obvious that the apparatus offers advantages over the ordinary flask for work such as seedling, alga, and liverwort culture on agar and other media and would also be suitable for germination tests and similar work.

The ones now in use were made in Germany, as it was at first found impossible to induce British manufacturers to undertake their production. This difficulty has been overcome, and it now remains to be seen whether the vessel commends itself sufficiently to mycologists and others to justify large scale production.

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The Initiation of the Dikaryophase in *Puccinia phragmitis* (Schum.) Körn.

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With Plates IX and X.

CONTENTS.

	PAGE
I. INTRODUCTION	403
II. MATERIAL USED	412
III. METHODS EMPLOYED	412
IV. EXPERIMENTAL WORK	413
V. CYTOLOGICAL WORK	416
The Compound Infection	416
The Haploid Monosporidial Infection	418
The Fertilized Monosporidial Infection	420
VI. DISCUSSION	423
VII. SUMMARY	428
VIII. POSTSCRIPT	429
LITERATURE CITED	431
EXPLANATION OF PLATES	437

I. INTRODUCTION.

EARLY VIEWS. The pycnidia of the rust fungi have been, ever since it was realized that they represent a constant stage in the developmental cycle of many species, regarded as connected, or as having at one time been connected, with sexual reproduction. Meyen (117), as early as 1841, originated the vague notion that they were male elements which might participate in some process analogous to fertilization. The discovery by Itzigsohn (89, 90) of similar bodies in the Lichens was made the basis of a similar hypothesis for this group, and Tulasne (161), although refusing to commit himself as regards their sexual function, originated the term 'spermatia' for the small spores seen in a number of Lichens and Ascomycetes. De Bary (63) borrowed the term for the pycnidiospores of the rusts, and was led to make further investigations with a view to settling

the question of their possible participation in sexual reproduction in this group; he pointed out (64) that although microscopic examination of aecidial pustules gave evidence of certain hyphae which emerged from stomata, and which might be regarded as serving as receptive organs, no fusion of spermatia with these could be observed, and the absence of any recognizable female element in the aecidium did not lend support to the hypothesis of a sexual function of the spermatia.

The discovery by Stahl (154) of fusion of spermatia with trichogynes in the Lichens may have strengthened, in certain quarters, the belief that a similar process of fertilization might be found in the Uredineae, but there remained, nevertheless, a sharp division of opinion on this point. For this was undoubtedly largely responsible the fact that in many rusts, Lichens, and other Ascomycetes, these spermatia were found, in certain circumstances, to germinate. Cornu (48, 49) made this discovery for a number of Ascomycetes, Möller for *Collema microphyllum* (118) and Cornu and Roze (50), Plowright (139), and Sappin-Trouffy (148), for various species of Uredineae. This capacity for germination seemed to many workers to be incompatible with the assumption that the spermatia were male reproductive cells, and they were led to the conclusion that they were non-sexual spores having a conidial function. Brefeld (31) was perhaps the chief protagonist of this school of thought; but De Bary, unwilling to desert the sexual hypothesis entirely, suggested that in such cases, where the female organs have been lost, the spermatia may have become secondarily adapted to perform a vegetative conidial function.

The fact remained, however, that many workers such as Fisch (76), Frank (77), and even Brefeld himself (30) were unable to induce the spermatia of certain Ascomycetes to germinate. Thaxter's ingenious experiment (159), in which he attempted to fertilize aecidial pustules of *Gymnosporium globosum* with spermogonial nectar, having failed to give any decisive results, the question of the possible sexual nature of the spermatia remained unanswered. In the Ascomycetes, Frank (77) reported fusion of spermatia with trichogynous hyphae in *Polystigma rubrum*; and Hartig (87), following the example of Stahl (153), suggested that similar sexual phenomena occur in the Uredineae. Klebahn (94, 97), although drawing attention to emergent trichogyne-like hyphae in various rusts, considered that definite proof of a fertilizing action of spermatia would be extremely difficult to obtain. Massee (116), on the basis of hand sections of living material, described the conjugation of an 'antheridium' with an 'oogonium' in the aecidium of *Uromyces Poae*, and Richards (146), at a later date figured a specially differentiated hypha at the base of the aecidium in several rusts. Neumann (122), however, confirmed the original observation of De Bary that a definite female element is lacking in the aecidium.

The first cytological studies. The advent of cytological technique was

destined to alter fundamentally the vague and unsettled viewpoints prevailing with regard to the phenomenon of sexuality in the Uredineae. Schmitz (150), in 1880, had reported, in *Coleosporium Campanulae*, the existence of paired nuclei in the mycelial cells and uredospores, and Rosen (147), thirteen years later, described binucleate aecidiospore chains in *Uromyces Pisi*.

Karyogamy in the teleutospore. Cytological evidence having a direct bearing on the sexual process in the rusts was brought forward by Dangeard and Sappin-Trouffy (62), who demonstrated that in the teleutospores of various rusts a fusion of the paired nuclei occurs; to this phenomenon they gave the name of 'pseudo-fécondation'. The simultaneous division of the paired nuclei in the rust mycelium was first observed by Poirault and Raciborsky (140), who originated the term 'conjugate division'. Dangeard (61) was of the opinion that the karyogamy shown to occur in the maturing teleutospore represents the true sexual act, and this opinion was shared by Sappin-Trouffy (148); the latter found that in a large number of rusts there are two kinds of mycelium, uninucleate and binucleate respectively, and that in the primary fructification a transition from the uninucleate to the binucleate mycelium takes place. The exact nature of this transition did not particularly engage his attention, since for him the fusion of the paired nuclei in the teleutospore represented the all-important sexual act. Wager (164) considered that in the higher fungi generally the fusion of a male nucleus with a female nucleus constitutes fertilization, and, while lacking the characteristics of a primary sexual act, replaces, and is physiologically equivalent to, the latter. A somewhat aberrant viewpoint was that of Groom (80), who regarded nuclear fusion in the teleutospore or basidium as a non-sexual phenomenon to which he applied the term 'deuterogamy'.

The findings of Sappin-Trouffy as regards the transition from uninucleate to binucleate mycelium in connexion with the formation of the primary fructification were confirmed for *Puccinia liliacearum* and *P. Bunii* by Maire (113, 115); but although he considered the binucleate phase to arise merely as a result of nuclear division, he differed from the opinion of Dangeard and Sappin-Trouffy in regarding the true process of fertilization as being constituted, not by the fusion of nuclei in the teleutospore, for which he invented the term 'mixie', but by the formation of the first synkaryons at the base of the primary fructification (114, 115). He furthermore stressed the fact that the synkaryon to which this apparently simple sexual process gave rise was equivalent to the diploid $2n$ nucleus of the higher plants (115). This viewpoint met with general acceptance, but the exact method by which the transition from the haploid to the diploid phase was effected remained for some years a mystery. Maire's hypothesis of the origin of the dikaryon by simple division of a cell nucleus without

subsequent septum formation seemed insufficient basis upon which to erect a scheme involving complete sexual differentiation, often in the course of four or five cell generations in the micro-forms, of nuclear elements destined to unite in the teleutospore.

Blackman's discovery. Arthur (18), in 1903, pointed out that the passage through the aecidial stage is marked by a definite reinvigoration of the fungus, very probably of a sexual nature. In the following year Blackman (26) brought forward evidence for the existence of a definite sexual process in connexion with the formation of the first binucleate cells in the Uredineae. This sexual process consisted of the migration, seen in fixed material of the young caeoma of *Phragmidium violaceum*, of the nuclei of mycelial cells into the specially differentiated fertile cells of the primordium. These fertile cells, which, in *Phragmidium*, form a palisade layer, were regarded by Blackman as female reproductive cells, and the degenerating sterile cells cut off at their tips as representing trichogynes which were, at a former phylogenetical period, concerned with the fertilization of these female cells by spermatia. This point of view led, of course, to the conclusion that the spermatia had lost all traces of their original sexual activity, and persisted merely as entirely functionless bodies, the role of which in sexual reproduction had become entirely usurped by an apogamous process occurring in the mycelium.

Christman's work. Christman (43), in the following year (1905), reported that in *Phragmidium speciosum* the binucleate phase originated at the base of the caeoma by the side-to-side fusions of neighbouring fertile cells, their nuclei thus coming to lie together in pairs in the 'two-legged' fusion cells. A similar phenomenon was observed by Christman also in the aecidium of *Uromyces Caladii*. This type of cell fusion was confirmed by Blackman and Fraser (27) for *Melampsora Rostrupi*, and the originally observed nuclear migrations for *Uromyces Poae* and *Puccinia Poarum*. Christman (44), in his study of the brachy-form *Phragmidium potentillae canadensis*, showed that in this species, with reduced life-cycle, cell fusions occur at the base of the primary uredosorus. He regarded the nuclear migrations of Blackman as artifacts, and the spermatia as functionless asexual conidia (45). It was not until Olive (129, 130) pointed out that the difference between nuclear migration and cell fusion was merely one of degree that the reconciliation between the observations of Blackman and Christman was completed.

Other investigators. In the years which followed, the findings of these two workers were confirmed and extended to a large number of species of Uredineae by the investigators here enumerated; a review of their individual researches cannot, for reasons of space, be here attempted: Olive (130, 131); Dittschlag (66); Kursanov (100, 102, 103); Pavolini (134, 135); Grove (81); Fromme (78, 79); Hoffmann (88); Werth and

Ludwigs (169); Moreau (120); Welsford (168); Colley (46); Dodge and Adams (72); Adams (1); Lindfors (110); Dodge (68); Ashworth (19); Cunningham (59); Lamb (105, 106); and Zeller and Lund (170).

Conditions in microforms. The researches of Blackman and Fraser (27), and of many of the workers listed above brought out the interesting fact that, whereas in the Uredineae possessing complete life cycles the initiation of the dikaryophase occurs (with the exception of certain instances to be mentioned later) at the base of the young aecidium, the microforms, in which the teleutospores are the sole propagative spores produced, show a surprising versatility as regards both the time and the place of the appearance of the first paired nuclei. Kursanov (103) pointed out that in such forms of reduced life cycle where the binucleate phase originates very early at an undetermined point of the developmental history the spermogonia are almost constantly lacking. A summary of the previously investigated microforms in this respect has been given by the present writer elsewhere (105).

The 'pre-aecidiospore' theory. Mention must be made in passing of a hypothesis put forward by Moreau (119) with regard to the question of the primitive function of the spermatia, at that time regarded by the majority of workers as functionless male cells. According to this theory, the sterile cells cut off from the tips of the fertile hyphae were to be regarded as the degenerate remains of 'pre-aecidiospores', which in a by-gone time constituted the female cells fertilized by the spermatia. Dodge (69), however, has emphasized the fact that exactly similar sterile cells are to be found in secondary uredosori, and has thus shown both the 'trichogyne' theory of Blackman and the 'pre-aecidiospore' theory of Moreau to be untenable.

According to Dodge's point of view (69) there were no known cases of sexual reproduction in the rusts, the phenomena of cell fusion and nuclear migration being purely substitute processes, comparable to the fusions occurring between auxiliary cells in the red algae.

Craigie's discovery. The discovery by Craigie (53, 54, 55, 56) of the existence of heterothallism in the Uredineae caused the problem of sexuality in this group to be regarded in a totally different light. The spermatia, for so many years relegated to the position of functionless bodies, assumed once more a major place in the consideration of the life cycle, and the entomophilous character of the spermogonia, upon which Ráthay (142) had made comment in 1882, was explained.¹ It was shown that in *Puccinia graminis* and *P. Helianthi* isolated pustules of monosporidial origin fail to produce

¹ Kursanov, in a paper in Russian published in 1915 (102), describes an attempt which he made to fertilize pustules of *Melampsorella Rostrupi* by applying spermogonial nectar to them in various ways. Owing to the technique of monosporidial inoculation being then unknown, this experiment, like that of Thaxter already mentioned, failed to give definite results.

acidiospores, although sterile wefts of mycelium are laid down in the leaf where aecidia would otherwise have developed. If the nectar from a number of pustules, however, was intermixed, aecidia made their appearance in a very short time. The transfer of spermogonial nectar from *one* monosporidial postule to a number of others resulted in the development of aecidia in approximately half of the latter, thus indicating that the heterothallism was probably of a bipolar nature.

Cummins (58) demonstrated by experiment that the fertilizing power in the spermogonial exudate of *P. graminis* resides in the spermatia themselves, and is not due to enzymes contained in the fluid in which they are embedded. In addition to a worker to be mentioned in a later paragraph, Mains (112) has recently shown that *P. Sorghi* is also heterothallic. Grove (82) draws attention to *Endophyllum Euphorbiae-silvaticae* as being a promising species for investigation in this respect.

Genetics of the rusts. Craigie pointed out that the discovery which he had made opened up a new field of study in the rusts, namely, that of hybridization; and since that time certain workers have obtained most interesting results along these lines. Newton, Johnson, and Brown (125, 126, 127, 128), Waterhouse (166), Stakman, Levine, and Cotter (157, 158), Levine and Cotter (108), Levine, Cotter, and Stakman (109), Newton, and Johnson (124), and Johnson, Newton, and Brown (92, 93) have proved that new physiologic forms of *P. graminis* may arise as a result of a technique of hybridization and 'selfing' on the barberry, and that certain other characters, such as spore colour, apparently obey Mendelian laws of inheritance. Cotter (52), and Stakman, Hines, Cotter, and Levine (156) offer evidence for the origin of hitherto unknown strains of stem rust in nature by hybridization and segregation on the barberry.

Certain points in the accounts of these investigators are of prime importance to those intending to make a study of the mechanism of the process of fertilization in the rusts. The first of these is the fact that Cotter (51), and Stakman, Levine, and Cotter (158), were able to isolate, following hybridization, more than one physiological strain of *P. graminis* from a single aecidial cup. Newton, Johnson, and Brown (126), however, although they reported the occurrence of several physiological forms in the same hybrid pustule, failed to obtain as a rule more than one such form from the same aecidium. Secondly, Newton, Johnson, and Brown (127) and Newton and Johnson (124) have brought forward striking evidence for the existence of the phenomenon known as cytoplasmic inheritance in the rust hybrids which they studied. In a large number of these, the pathogenic characteristics corresponded more closely to those of the form from which the acidiospores were isolated than to those of the form which had supplied the exudate used in fertilization. This difference in the offspring of reciprocal crosses was found to persist through all subsequent generations, and

was hence not subject to segregation and recombination like the other pathogenic characters involved in the crosses; it may best be explained by the assumption that the cytoplasm of the 'maternal' pustule which gives rise to the aecidiospores forms the major part of the plasmic content of the latter, and, carrying, as it may from analogy with the higher plants be expected to do, a certain portion of the heritable characteristics, impresses upon them such of a preponderantly 'maternal' nature. The significance of this feature in the consideration of the mechanism by which fertilization in the rusts is effected will be referred to in a later part of this paper.

It becomes obvious from the foregoing considerations that in spermatial fertilization the nuclei of the spermatia must, in some way, come to be associated with those of the fertilized pustule in the form of dikaryons which are ultimately enclosed in the aecidiospores. The attempts already made to elucidate this process will now come under brief consideration.

Hanna's work. Hanna (86) showed that in *P. graminis* the unfertilized monosporidial pustules are entirely uninucleate; near the lower epidermis of the barberry leaf numerous sterile wefts of haploid mycelium appear, which are the primordia of eventual aecidial cups.¹ About 48 hours after the transferal of spermogonial nectar, according to Hanna, the nuclei at the base of each aecidial weft become enlarged, and neighbouring hyphal cells then fuse in pairs, as described by Christman, to give rise to binucleate fusion cells which proceed to cut off chains of aecidiospores. It was observed by Hanna that the spermatia in some cases form germ tubes, and he considered that the haploid hyphae thus produced grew down through the leaf to the wefts, where they fused with cells of opposite 'sex'.

Buller's Theories. Buller (37) conceived the process of diploidization in a heterothallic rust to take place in one of the following ways: (a) in a bisporidial infection the two haploid mycelia of different 'sex' may intermingle so that they eventually become arranged in a mixed fashion in the spore bed of the young aecidium, where cell fusions or nuclear migrations occur between them; or, as an alternative, no appreciable intermingling may take place, but nuclei may be interchanged between the two mycelia, so that eventually in the hyphae forming the aecidial rudiments nuclei of both 'sexes' are present, which then become cut off in individual cells. Cell fusions and nuclear migrations then occur between these cells. (b) In the case of spermatial fertilization, the spermatia deliver into the mycelium their nuclei, which, by dividing and migrating, diploidize every cell destined to produce a spore chain.

Allen's Work. Allen (4, 5, 6, 7, 8, 9, 10) has made a very thorough

¹ It is of interest in this connexion to note that, as Jackson (91) points out, such sterile hyphal wefts, probably resulting from an unfertilized monosporidial infection, were described by Olive (132) as far back as 1911.

series of studies of heterothallism in *P. graminis*, *P. coronata*, and *P. trititina*. She confirms Hanna's report that the sterile aecidial wefts are predominantly uninucleate, with the exception of *P. coronata*, in which, at a certain stage in the development of the unfertilized pustule, multinucleate cells appear in the majority of the hyphal wefts. In all cases, if fertilization is not achieved, the sterile primordia, after having undergone the primary differentiation into an outer part of large empty cells and an inner part of small plasm-rich cells, die without producing spores. In a fertilized infection, however, binucleate cells may be seen in various parts of the thallus; in the walls of the spermogonia, in the vegetative mycelium and, ultimately, in the aecidial fundament. Shortly after fertilization, the spermogonia cease to produce spermatia, and their exudate dries up. Some evidence was obtained for each of a variety of ways in which the spermatia might become associated with the mycelium of the pustule; by fusion with the periphyses; by fusion with hyphae protruding from stomata or forcing their way out between epidermal cells; by the germination of spermatia to give hyphae which grow down through the ostioles of the spermogonia; and by fusion of spermatia in pairs and the growth of the presumably diploid hyphae thus produced towards the spermogonial openings. Cell fusions of the Christman type were observed, but by no means in the profusion in which they might be expected were they the sole means by which the initiation of the dikaryophase was effected; and the 'two-legged' cells, often considered by the earlier investigators as sufficient proof of cell fusion, were shown to originate in a number of cases at least by the downward proliferation of the basal cells.

Andrus' work. A very original hypothesis was originated by Andrus (16, 17), who maintained that the fertile cells at the base of the acidium in *Uromyces appendiculatus* and *U. Vignae* are actually 'egg cells' which possess hyphal prolongations ending on the surface of the leaf as 'trichogynes'. He claimed that spermatia fuse with these 'trichogynes', and, in some cases, pass into the ruptured hyphae. In his second cytological investigation on these rusts, he discovered that the nuclei of the spermatia could be distinguished from those of the fertilized pustule by a differential staining reaction, so that they could be easily traced in their wanderings through the mycelium. According to Andrus, the mere presence of the fertilizing spermatial nuclei in the aecidial primordia is sufficient to cause the nuclei of the 'egg cells' to migrate into contiguous cells; these 'accessory' migrations and cell fusions 'would appear to have a nutritive effect and would support later proliferation of the multinucleate basal cell'. Andrus' description, together with his use of the term 'accessory cell fusion', indicates that he visualizes for the rusts a sexual process analogous to that found in the red algae.

Rice's work. Rice (145) has made some interesting cytological

observations on a number of rusts, but the fact that the infections which she studied were not of proved monosporidial origin detracts considerably from the value of her data. She also draws a parallel between the sexual process known for the red algae and that surmised for the rust fungi.

Behaviour of spermatia. The first definite proof of fusion of spermatia with the thallus of the fertilized pustule was brought by Craigie (57), who showed that in *P. Helianthi* the spermatia, after fertilization, become attached laterally by short bridges to irregular hyphae emerging from the ostioles of the spermogonia. Pierson (186) described a similar fusion of spermatia with filamentous hyphae in *Cronartium ribicola*, consequent upon mixing of nectar. Although some of the attached spermatia were devoid of contents, no cases of migration of nuclei into the hyphae were observed. CUMMINGS.

Allen (13) now figured spermatia of *P. Sorghi* united with the spermogonial periphyses of a fertilized pustule, and showed that their nuclei enter the mycelium via these periphyses. Under certain conditions, she claims, spermatia may possibly germinate and grow down through the ostiole into the spermogonium. In *Melampsora Lini* Allen (11, 12) found evidence for yet another mode of spermatial fertilization. In this heterothallic rust there were indications that the spermatia, after being transferred to an infection, effect entry into epidermal cells by penetrating the outer cell wall; after moving or being carried to the inner wall, the few spermatia which have survived the experience apparently penetrate it and give rise to hyphae which grow into the intercellular spaces. The cell fusions which Fromme (78) had shown to be such a conspicuous feature of this rust were confirmed, and their occurrence is stated by Allen (12) to be 'presumptive evidence that the hyphae of spermatial origin maintain an independent existence up to this point'.

Action of diploid mycelium. Brown (34) has made the interesting discovery that monosporidial pustules of *P. Helianthi* can be diploidized in the leaf by an already diploid mycelium derived from the sowing of uredospores of the same species beside them. The process involved is clearly similar to that reported by Buller (37) in the Hymenomycetes.

Nature of cell fusions. We are thus left with two hypotheses with which to explain the cell fusions which earlier investigators have shown to occur with such regularity at the base of the aecidial fundament: (a) that they are, as Allen (12) seems to infer, a result of the preservation of the spermatial mycelia as distinct entities up to the base of the aecidial weft, or (b) that they are actually comparable to the secondary fusions of auxiliary cells known to occur in the red algae, as suggested by Dodge (69), Andrus (17), and Rice (145).

II. MATERIAL USED.

Puccinia phragmitis (Schum.) Körn. is a heteroecious eu-form, having its uredo- and teleuto-stages on *Phragmites communis*, and its spermogonia and aecidia, as was first shown by Plowright (187, 188) on species of *Rumex* (excluding *R. Acetosa*) and *Rheum*. The teleutospore material used for the present study was collected in the autumn of 1933 on the banks of the river Main at Randersacker, near Würzburg, Germany. The seeds of *Rumex crispus* used to raise plants for infection were also obtained from the neighbourhood of Würzburg.

III. METHODS EMPLOYED.

Inoculation. The inoculations were made in spring, as soon as samples of the teleutospore material, which had been conserved throughout the winter out of doors in stoneware trays, showed a good percentage of germination in the damp chamber. Rusted *Phragmites* leaves were tied to pads made up of pieces of millboard impregnated with paraffin wax and wrapped in several layers of damp filter paper, well sprayed with an atomizer, and secured by the pads to which they were attached to the lower inner surfaces of five-litre pickle jars lined with wet filter paper. These jars were then kept inverted over water for twenty-four hours to allow germination to commence. At the end of this period one jar, as a sample, was placed over a flower-pot containing earth, over which was secured a clean microscopic slide in a horizontal position. The period required for a fairly sparse inoculation was readily determined by microscopic examination of this slide an hour later.

All the jars were now inverted over flower-pots containing young plants of *Rumex crispus* raised from seed for the purpose, and left thus for the calculated period, the connexion between jar and pot being made as airtight as possible with saturated cotton wool. Half an hour was found to be sufficient for the inoculation of each pot; the jars were then removed, and the pots placed underneath the benches of a *Hymenophyllum* house, where the atmosphere was practically saturated with water vapour, at a day temperature of about 18° C. Here they were left for twenty-four hours to permit the sporidia to germinate and penetrate the leaves of the host plants, and were thereafter transferred to the shelves of an ordinary unheated greenhouse.

Mass inoculations were also made by placing saturated teleutospore material in glass cells and applying these to young *Rumex* leaves; when left thus for two hours, an extremely dense inoculation resulted, and the plants were then kept over a period of twenty-four hours under inverted glass jars lined with wet filter paper.

Protection against insects. When the infections appeared on the leaves the pots were covered, as a protection against insects, with cages specially constructed out of cellophane and fine meshed muslin; these were, however, found to be inefficient in keeping out certain minute flies, and resort had to be made to the enclosure of the individual leaves in large glass boiling tubes, the ends of which had been knocked out and covered over with very fine voile cloth to allow of ventilation. These tubes, the mouths of which were sealed round the leaf petioles by cotton wool plugs, were found to perform their function satisfactorily.

Cytological technique. Material at the required stages of development was cut out of the leaf, subdivided if necessary, placed into tubes containing fixative, and immediately exhausted with an air pump until it sank in the solution. Two fixatives were used: Bouin's solution in the modification suggested by Bauch (24), and Flemming's stronger solution diluted with an equal quantity of water. The latter was found to give slightly better results than the former. The material, after remaining twelve to twenty-four hours in the fixative, was transferred by the usual series of stages to paraffin wax, impregnated, imbedded, and microtomed into sections from 5 to 10 μ in thickness.

The stains used were: Breinl's combination of safranin, polychrome, methylene blue, and orange tannin, as described by Gwynne-Vaughan and Barnes (84), Heidenhain's iron alum haematoxylin (counterstained with Congo red), and the simultaneous eosin-methylene-blue combination of Mann, mentioned by Bauch (24). Of these methods, iron alum haematoxylin and Congo red was found to give the clearest differentiation, although Breinl's combination also gave very good results; Mann's staining technique, although poor for the finer cytological structure, possessed the advantage of being extremely transparent, and was hence well suited for very thick sections. In mounting the preparations, use was made of the Bayer product 'Caedax', which was found to be in all respects superior to ordinary Canada balsam.

IV. EXPERIMENTAL WORK.

Sporidia. The sporidia of *Puccinia phragmitis* are shot off singly from the promycelium; about half a minute before the projection, a droplet of fluid is formed at the junction of each sporidium with its sterigma, after the manner described by Dietel (65), Weimer (167) and Buller (35). Sporidia shot off in the damp chamber, and adhering to the cover glass, may germinate to form either secondary sporidia or germ tubes.

Infections. Five days after the inoculation minute ring-shaped purple-red spots, 1 to 1.5 mm. in diameter, made their appearance on the leaves of the infected plants. In twenty-five of the pots a large number of

undoubtedly monosporidial infections were numbered with Indian ink, and covered with the specially constructed cages mentioned in the preceding section; a number of monosporidial pustules in the remaining pots were marked in a similar way, but left exposed. By the following day the infections had formed sweet-smelling spermogonia, which gradually exuded droplets of spermatial nectar.

Insects. The examination, in the course of the next few days, of the caged pots showed that the muslin and cellophane cages used were insufficient to keep out certain minute flies with which the greenhouse abounded, and which apparently fed on the spermogonial nectar. Accordingly, without loss of time, these cages were replaced by the glass tubes previously mentioned; these were found to be effective in preventing the entry of even the smallest insects.

Fertilisation. The nectar of the unprotected marked pustules was now thoroughly intermixed with a camel-hair brush; seventy-two hours afterwards the spermogonia were seen to have become dried up, and in five days' time all the pustules, without exception, had produced open aecidia, mostly on the under surface of the leaf. These aecidia were white in colour, and encircled by a zone of crimson-coloured leaf tissue (Pl. IX, Fig. 1). Many of the compound infections, and also certain of the monosporidial infections, in the glass tubes simultaneously developed aecidia, the latter doubtless as a result of insect activities referred to above. A number of the double pustules were seen to form aecidia at first along the line of coalescence. Monosporidial pustules mixed at a later date, nineteen days after inoculation, developed open aecidia forty-eight hours after mixing; hence the process of fertilization is accomplished in considerably less time in older than in younger infections. The nectar of several of the monosporidial pustules in the glass tubes was also intermixed, to serve as a control; these pustules all gave rise to aecidia in a short time, proving that enclosure in the ventilated tubes had no effect on the development of aecidia.

Sterile pustules. Fertilized pustules, as a rule, cease to increase in diameter at the time when spore formation sets in; but an isolated monosporidial infection increases steadily in size by peripheral growth until death eventually ensues. On the tenth day after inoculation, the formation of sterile aecidial wefts became apparent to the naked eye as translucent points extending over an area greater than that covered by the spermogonia. As the centrifugal development of the unfertilized pustule proceeded, the original spermogonia in the centre ceased to function, and were replaced by others at the periphery. Old sterile infections attained a diameter, in many cases, of over 2 cm., and by reason of their crimson colour, due to the development of anthocyanin in the leaf, presented a somewhat striking appearance. Pl. IX, Fig. 2, represents such a sterile, unfertilized pustule

photographed by both reflected and transmitted light, twenty-eight days after inoculation; the aecidial wefts can be seen as lighter coloured points, which are actually more translucent areas.

Heterothallism. The leaves of *R. crispus* are short-lived, and hence the final examination of all pustules was carried out exactly one month after the infection. The results were as follows: (a) of 104 monosporidial infections whose nectar had been mixed, all had developed aecidia; (b) of 157 monosporidial infections protected, as far as possible, from insects, 54 had developed aecidia, doubtless due to the small flies which gained access to the muslin cages; (c) of 49 double pustules (two coalescent monosporidial infections), 34 had developed aecidia; (d) of 38 multiple pustules (three or more coalescent monosporidial infections), all had developed aecidia. This is, then, fairly clear evidence that *P. phragmitis* is heterothallic, and it is probable that with complete exclusion of insect visitors all unmixed monosporidial pustules would have remained sterile.

Other experiments. The following experiments were also carried out:

(a) Nectar from a large number of pustules was applied to one side only of two monosporidial infections; the latter subsequently developed aecidia on the side thus treated, although the sharp differentiation was soon lost by the spreading of aecidial development throughout the whole pustule. This was a repetition and confirmation of an experiment originally made by Craigie (55) with *P. graminis* and *P. Helianthi*.

(b) Spermatogonial nectar of mixed origin was brushed on to the under side of a cover glass forming the upper part of a damp cell, and microscopically examined at intervals during the next few days for evidence of any conjugation of spermatia such as suggested by Allen (5) for *P. graminis*. Under these conditions many of the spermatia were seen to put out short germ-tubes, but in only one instance, among the thousands of spermatia examined, was any appearance suggestive of fusion of spermatia in pairs seen; in this isolated case two spermatia were observed to be apparently fused by their ends. This cannot be regarded as convincing evidence for the regular occurrence of such a phenomenon.

(c) A mixture of germinating sporidia and spermatial nectar was put up in a damp chamber on the under side of a cover glass, and periodically examined under the microscope for any evidence of fusions between sporidial germ-tubes and spermatia, or among the sporidia themselves; no appearances hinting at fusions were, however, observed. This point will be discussed in a subsequent section.

(d) To ascertain whether the spermatia themselves are capable of causing infection, nectar of mixed origin was applied to certain marked places on the upper surfaces of young leaves of a healthy *R. crispus* plant, which was kept over water under a bell jar for several days afterwards;

but at no time did any trace of infection appear. This bears out the results of the experiments of Klebahn (94, 95, 96) and Plowright (139), who were likewise unable to produce infection by means of spermatia.

Plentiful material of both fertile and sterile infections at various stages was removed from time to time for fixation, and formed the basis for the cytological study presented in the following sections.

V. CYTOLOGICAL WORK.

The Compound Infection.

Infection. The sporidia apparently lie on the surface of the leaf for at least twenty hours before penetrating the cuticle, for in material fixed at that time after inoculation no traces of infection were seen. In portions of the leaf fixed forty-eight hours after inoculation, however, numerous cases of invasion of the epidermal cells were apparent. The process of infection is similar to that described by Waterhouse (165) and Allen (5, 9) for *Puccinia graminis* and *P. coronata*. Primary infection hyphae are usually formed inside the epidermal cells, and give rise to runners which grow down into the mesophyll.

Development. The hyphae formed in the leaf-tissue possess one to six nuclei per cell (Pl. X, Fig. 5). The branches appear to arise in practically all cases immediately behind the septa, and become septated off close to their point of origin, a point which was noticed also by Fromme (79) in *P. Claytoniana*. The nuclei take the form of deeply staining nucleoli surrounded by clear haloes, and agree in this respect with those of a large number of Basidiomycetes and Ascomycetes. Ninety-six hours after inoculation the mycelium has traversed the whole thickness of the leaf, and on the fifth day after inoculation spermogonia are laid down, the majority on the upper surface of the leaf. Simultaneously globules of red anthocyanin pigment become apparent inside the host-cells, and increase in size and number as the pustule becomes older. Vacuolate buffer cells, as described by Allen (5) in *P. graminis*, perform the function of raising the epidermis above the developing spermogonia.

Spermogonia and spermatia. On the sixth day the spermogonia are fully formed, and have commenced to give rise to spermatia: the spermatophores are regularly uninucleate, and abstrict from their tips in succession the spermatia, the cytoplasm of which stains somewhat densely, but usually not so densely as to obscure completely the single nucleus situated, as a rule, towards one end of the spermatium (Pl. X, Fig. 6). Whether the heavy staining reaction of the spermatial cytoplasm is due to stored food substances was not determined; if this is the case, *P. phragmitis* would seem to be peculiar in this respect, as previous investigators, in describing

the structure of rust spermatia, are agreed that they carry little or no reserve food material.

Aecidial primordia. Examination of eight-day compound infections shows that the formation of aecidial primordia has begun. These are formed by the convergence of plasm-rich, mostly multinucleate hyphae towards central points below the lower epidermis of the leaf. A few primordia usually differentiate also on the upper surface. The subepidermal cells are forced apart, and the epidermis raised, by the strong mycelial growth. Very soon the fundement is seen to become differentiated into an inner zone of richly protoplasmic cells, the so-called 'fertile layer', and an outer zone of considerably swollen and highly vacuolate 'sterile cells', which are actually the terminal series of cells belonging to the more or less parallel hyphae composing the primordium.

Cell fusions. In eight-day stages of compound infections, i.e. infections derived from inoculation with a large number of sporidia, and which may hence be regarded as containing mycelia belonging to both groups necessary for the formation of aecidia, very numerous cases of cell fusion of the Christman type may be seen in the fertile tissue of the aecidial fundaments. In many preparations practically every section on the slide showed at least one cell fusion; three representative examples have been drawn in Pl. X, Figs. 9, 10, and 11. Fig. 9 shows a binucleate fusion cell; its two nuclei have already undergone conjugate division to form a second binucleate cell, at the summit of which the two degenerating sterile cells originally belonging to the two fusing hyphae may be seen. In Fig. 11 one of the partners in the fusion is multinucleate, so that a fusion cell with more than two nuclei would have been produced. A striking characteristic of these fusion cells seen in polysporidial infections is the dense and granular nature of their protoplasm; this, as will be seen later, appears to be an index of the syngamic compatibility of the cells concerned, and forms a valuable criterion in judging of the syngamic or vegetative nature of certain cell fusions.

Origin of dikaryophase. It is clear, then, that in pustules of polysporidial origin the initiation of the dikaryophase occurs exactly as described by Christman, Blackman and Fraser, and many other investigators before the discovery of the function of the spermatogonia in the Uredineae. Craigie (53, 54) made the suggestion that diploidization in compound pustules of *P. graminis* and *P. Helianthi* may occur in this manner; Allen (5, 9) was unable to find conclusive evidence of cell fusion in polysporidial infections of *P. graminis* and *P. coronata*, and considers that the sporophytic growth may originate either within the leaf by hyphal fusions or on the surface of the leaf by the agency of the spermatia. As far as *P. phragmitis* is concerned, however, diploidization in the compound infection occurs by the former method, as suggested by

Buller (37); the phenomena attendant on spermatial fertilization are, as will be seen later, of an entirely different nature.

The Haploid Monosporidial Infection.

Spermogonia. In unfertilized monosporidial pustules, as previously explained, the first-formed spermogonia in the centre eventually die, and are replaced by new spermogonia at the periphery. In *P. phragmitis* the spermogonia are flask-shaped, 78–90 μ in diameter, and provided with a brush of periphyses round the ostiole. These periphyses contain one or two small nuclei, and are bluntly pointed or bulbous at their tips (Pl. X, Figs. 7 and 8). Their protoplasm is thin.

Aecidial wefts. Ten days after inoculation, haploid aecidial wefts are formed in large numbers. At first the differentiation into an inner layer of richly protoplasmic cells and an outer layer of vesicular cells is very definite (Pl. IX, Fig. 3); but very soon the cytoplasmic contents of the inner cells also become scanty and vacuolate. This is an indication that the infection is definitely haploid and unfertilized, and it is advisable to defer cytological observations on the sterile monosporidial pustule till this stage, as the danger of unwittingly describing accidentally fertilized infections is thereby eliminated.

Vegetative cell fusions. Multinucleate cells containing two to five nuclei are common in the sterile aecidial tissue at this time; rare cases of cell fusion in the inner layer may also be seen if careful search is made. But these cell fusions differ essentially from those seen in compound pustules by the fact that the hyphae involved present an undernourished appearance, with scanty and vacuolate protoplasm. Two such cases are figured in Pl. X, Figs. 12 and 13; it will be seen at a glance that they cannot be compared with the definitely syngamic cell fusions occurring in the compound pustule. They are, in fact, nothing more than purely vegetative hyphal anastomoses, and are not involved in any way in reproduction. Buller (38), in his review of the literature on hyphal fusions in the fungi, points out that they are common to practically all groups, and that their formation has nothing whatever to do with the particular syngamic condition of the mycelia concerned; they are of value in allowing for close co-operation and transport of food materials between neighbouring mycelia. Gwynne-Vaughan (83) regards the tendency to hyphal fusions in the Basidiomycetes as being presumably due to a nutritive stimulus, and purely vegetative anastomoses between uredo-mycelia of various rusts have been observed by Büsgen (40), Voss (163), and Allen (3). There is, then, no reason for doubting the occurrence of agamic vegetative hyphal fusions in sterile monosporidial pustules of *P. phragmitis*.

Structure of aecidial wefts. Apart from these cell fusions, the struc-

ture of the aecidial weft at this stage is relatively simple; it is composed of crowded convergent hyphae, of which the terminal cells have, to a variable number, become greatly swollen and vesicular. These hyphae exhibit very profuse branching, so that, unless one is dealing with a median section through the primordium, it is practically impossible to make out the relationships of the very crowded cells of which it is formed.

Evacuation of mycelium. The vacuolization of the inner layer of cells proceeds apace as the pustule becomes older, and towards the twentieth day after inoculation they swell up and become morphologically completely indistinguishable from the cells of the outer layer. In Pl. IX, Fig. 4, is a microphotograph of a section through such a sterile weft in an unfertilized monosporidial pustule fixed twenty-two days after inoculation. All the primordia in the infection present this appearance, with the exception of those newly formed at the extreme periphery; the latter, however, soon go over to the vacuolate condition as later primordia are successively formed farther out from the centre.

At first sight these completely vacuolated aecidial fundaments appear to be dead; but on examination under the higher powers of the microscope their cells are seen to have retained a thin lining of protoplasm, and in many of them the nucleus can be observed attached by the cytoplasm to the cell wall, and, apparently, quite intact (Pl. X, Fig. 14). The vegetative hyphae in the mesophyll have also become highly vacuolate, but retain their nuclei, still living, slung by protoplasmic strands (Pl. X, Fig. 15). In such vacuolated cells the structure of the nucleus can be better made out than when surrounded by richer protoplasm; although the nucleolus alone takes up the basic stain, the nucleus as a whole is seen as a sharply defined vesicle.

This striking evacuation of the mycelium in the inner part of the infection can be readily understood when it is realized that the unfertilized pustule is undergoing continuous centrifugal growth and differentiation of an enormous number of spermogonia and aecidial primordia, with a consequent drain on the cytoplasmic resources. Buller (38) has shown that the protoplasm of the vegetative mycelium of *Rhizopus nigricans* is rapidly evacuated during the formation of sporangiophores and sporangia, and Liu (111) reports that in the apple rust, at the time when the spermogonia are in full development, the ground mycelium is difficult to distinguish owing to its depletion of protoplasm by the spermogonia; when the latter have concluded their activities, the mycelium becomes again replenished and proceeds to initiate the aecidium. In the present case, the thallus of the sterile monosporidial infection remains, with the exception of its actively growing outer edge, in this extremely vacuolated condition until fertilization or death ensues.

The Fertilized Monosporidial Infection.

Behaviour of spermatia. Following upon spermatial fertilization of a monosporidial pustule of *P. phragmitis*, certain of the transferred spermatia fuse with the periphyses of the spermogonia. Although a large amount of fertilized material was examined cytologically, only two cases of such fusion were seen in the preparations, and have been drawn in Pl. X, Figs. 16 and 17. The observation of Allen (13) in *P. Sorghi*, that over 95 per cent. of the spermatia which have become attached after fertilization wash off in the processes of fixation, staining, &c., would seem to hold good for *P. phragmitis* also. Both the cases seen here were in material fixed forty-eight hours after fertilization. In the first case (Fig. 16), the spermatium has fused by a lateral bridge with the periphysis; it has also, apparently, given rise at its apex to a very short germ-tube, from which the protoplasm has now become retracted. Unfortunately the staining of this preparation is not sufficiently good for the nuclei of this portion to be made out. Fig. 17 represents the fusion of a spermatium with *two* adjoining periphyses, one being of the bluntly pointed, and the other of the bulbous type. The spermatium has undergone a certain amount of elongation, and is noticeably vacuolate. In this case certain bodies which may be interpreted as nuclei are seen; one is in the spermatium, one in the longer connecting bridge, and seven in the lower periphysis near its tip. These bodies agree in structure with nuclei, but seem to be optically somewhat more refractive; and as in size they approach the boundary of microscopic resolution, their interpretation as nuclei should not, perhaps, be accepted without reserve. Their size, however, corresponds closely to that of certain undoubted nuclei about to be described. No hyphae protruding through stomata or between epidermal cells have been observed, and it is probable that in *P. phragmitis* the spermogonial periphyses are the sole receptive organs for spermatia.

Spermatial nuclei. In ten days old monosporidial pustules fixed twenty-six hours after fertilization the aecidial wefts are still in the haploid condition, and present the appearance shown in Pl. IX, Fig. 3. But examination of the vegetative mycelium brings to light the fact that in many of its cells minute nuclei, $0.75-1.5\mu$ in diameter, are present alongside of the original thallus nuclei, which at this stage possess a diameter of $2.0-2.5\mu$. As these small nuclei are only seen in material which has been fertilized by spermatia, one may deduce that they are spermatial in origin. Pl. X, Fig. 18, represents part of a vegetative hypha containing two thallus nuclei and three spermatial nuclei: as shown here, the latter are often to be found closely paired, a condition hinting at their multiplication by division within the thallus cells. In material fixed twenty-four hours later, the inner layer of the aecidial wefts has become very multinucleate,

its basal cells in particular being crowded with large numbers of spermatial nuclei. On examination of the vegetative mycelium at the base of the aecidial wefts, many of the hyphal septa are seen to have become partially dissolved from one side (Pl. X, Fig. 19), thus allowing of the migration from cell to cell of the spermatial nuclei.

Fusion cells. Occasional fusion cells may be observed in the aecidial primordia at this stage, but it is fairly obvious that they are of the purely vegetative type mentioned in the preceding section, and have probably originated before fertilization occurred. Such fusion cells are figured in Pl. X, Figs. 20, 21 and 22. In Fig. 20 the fusion cell contains only the two original thallus nuclei, and hence is definitely not of a syngamic nature: the fusion cells in Figs. 21 and 22 have already been reached by spermatial nuclei: the interpretation of Fig. 22 as a fusion cell is open to doubt, as only one thallus nucleus can be seen in it. In Fig. 21 the adjoining cell walls have become dissolved in two places; whether this is due to an original vegetative cell fusion or represents a subsequent breaking down under the influence of the spermatial nuclei cannot be determined. It is significant, however, that fusion cells are seen in the fertilized aecidial fundament with about the same frequency as in the unfertilized aecidial fundament.

Diploidization. Fig. 23, on Pl. X, shows a branched hypha of the primordium in process of diploidization; its end cell has become swollen and vacuolate to form one of the sterile cells of the outer layer. Four spermatial nuclei have entered from the vegetative mycelium, and would, no doubt, have passed down into the adjoining fertile cells. When the diploidization of the basal cells has been completed, a number of the small spermatial nuclei may be seen left behind in the ground mycelium, where they undergo degeneration as evacuation by the developing aecidia commences; this, together with the very multinucleate condition of the spore-chain mother-cells, may be a result of the transfer of a large amount of spermatial nectar in the process of experimental fertilization. It is certain that under natural conditions the number of spermatia transferred is relatively small, and the numerical relationship between thallus nuclei and spermatial nuclei is hence probably more balanced. The spore-chains, which arise from the outer cells of the inner fertile layer, are, however, mainly binucleate, although multinucleate spore-chains are by no means rare; an indication as to how this regulation probably occurs is given in Pl. X, Fig. 24, in which, in an originally trinucleate cell of the aecidial primordium, two nuclei only are undergoing conjugate division, while the third remains in the resting condition.

Although spore-chain mother-cells with unequally sized nuclei may often be observed, the growth of the spermatial nuclei to the size of those of the basal cells appears to be accomplished with great rapidity.

In fourteen days old monosporidial pustules which have been fertilized 120 hours previously, the aecidia have already burst the epidermis, and are liberating their spores.

Fertilisation of the aged pustule. It was seen in the foregoing section that towards the twentieth day after inoculation a general evacuation of the vegetative mycelium and aecidial wefts of the unfertilized monosporidial infection sets in. Yet experiment has proved that not only is such a pustule capable of being fertilized, but that the time elapsing between transfer of nectar and development of open aecidia is also considerably less than in the case of younger infections. Microscopic study of a twenty-days old monosporidial infection fixed twenty-four hours after fertilization has brought the following facts to light: as in all cases, the spermatial nuclei apparently reach the aecidial wefts by way of the already existing thallus mycelium; as far as could be ascertained no new hyphae are laid down.

The proximity of the spermatial nuclei appears to effect the regeneration of the cytoplasm in the hyphae which they have entered. Evidence of this can be seen in the already diploidized aecidial primordium, where certain of the swollen inner cells along the paths of diploidization are observed, immediately after the formation of spore chains has begun, to have acquired densely protoplasmic contents in which lie the residual nuclei (Pl. X, Fig. 25). The basal cells of the spore chains are formed from certain primordial cells in the midst of the fundament; behind these is a layer of inner primordial cells, some of which have served as pathways for the diploidizing nuclei. In this cell-layer, although dissolution of certain cell-walls has taken place, the cells through which diploidization has occurred are obviously none other than the original, previously vacuolated cells of the aecidial weft; their intimate and undisturbed relationship with their vacuolated neighbours is apparent upon focusing up and down. There is no evidence of crushing or disturbance of those primordial cells which have remained in the evacuated condition, as would have certainly ensued had the richly protoplasmic 'pathway cells' belonged to external hyphae forcing their way up to the level where spore-chain mother-cells are formed.

In material fixed a few hours later the 'pathway cells' have become once more entirely evacuated of their contents by the developing spore-chains, and forty-eight hours after fertilization the aecidia have already ruptured the epidermis and commenced to liberate spores. It remains unexplained why the fertilization of the old evacuated thallus, entailing protoplasmic regeneration of all the cells through which the spermatial nuclei pass, should be accomplished so much more rapidly than in the case of a younger, still densely protoplasmic mycelium.

VI. DISCUSSION.

Time of syngamic reaction. From the foregoing account emerges the fact that diploidization in *Puccinia phragmitis* can occur in two different ways: (a) by fertilization with spermatia, and (b) by the intermingling of compatible mycelia in a polysporidial infection, followed by cell fusions between these in the aecidial primordia. In the case of diploidization by the latter method, it is of interest to note that the syngamic reaction between the compatible mycelia does not occur until a certain point in their development is reached; as seen in the experimental section, fusions between the germ-tubes of sporidia and between sporidia and spermatia do not occur. The mycelia in the leaf seem also to be incapable of syngamic reaction until aecidial fundaments have been laid down. This delay in syngamic potentiality is not without parallel in other groups of fungi. Burgeff (39) found that mycelia of *Phycomyces* were unable to react sexually until they had reached a certain age, and Bauch (23) reports that in the *macrospora* variety of *Ustilago longissima* the first binucleate sporidia ('Fruchtträger'), although their two nuclei belong to opposite reproductive groups, show no evidence of bridging 'horseshoe' fusions between their two cells, copulation being manifested only between the uninucleate sporidia subsequently abstracted from them. Brodie (32) has shown that very young haploid mycelia of *Coprinus lagopus* are not syngamically reactive, and continue to produce oidia for some time even if growing in contact with mycelia of a complementary compatibility group.

The position in *P. phragmitis* appears to be comparable to that in *Ustilago Zeae*, investigated by Rawitscher (143) and Stakman and Christensen (155), and in *Doassansia Sagittariae* investigated by Rawitscher (144); here there is no copulation between sporidia, the formation of the first dikaryons apparently taking place by hyphal fusions within the host plant shortly before chlamydospore production.

Passage of nuclei. Most of the recent workers on the cytology of the rusts are agreed that diploidization following spermatial fertilization occurs in most cases by way of the already existing mycelium of the fertilized infection, and that this process involves the passage of nuclei along the hyphae to the site of the formation of the first dikaryons. Lehfeldt (107) has shown that diploidization in *Typhula erythropus* is accompanied by the partial breaking down of the septa to allow of the passage of the diploidizing nuclei. Buller (36, 37) originally suggested that the same phenomenon occurs also in the heterothallic Hymenomycetes which he investigated, but later, as a result of his studies of protoplasmic translocation, maintained that the diploidizing nuclei might pass through the septal pores, without any breaking down of the cell-walls (38). In *P. phragmitis*,

however, there is fairly good evidence that a certain amount of septal dissolution does occur, very much after the fashion originally described by Lehfeldt.

Is the ground mycelium diploidized? Another question which arises in connexion with spermatial fertilization is whether every cell through which the spermatial nuclei pass becomes diploidized. As seen in the foregoing study, a considerable number of spermatial nuclei actually remain behind in the ground mycelium after diploidization of the aecidial primordia has been completed, but, as explained, this is probably in the nature of an artifact due to fertilization with a superabundance of spermatial nectar, and it is doubtful whether this would be the case in circumstances of natural fertilization by insects. It is certain, at any rate, that the spermatial nuclei left behind in the ground mycelium do not enter into a close conjugate relationship with the thallus nuclei, and, in view of the subsequent complete evacuation of the vegetative mycelium by the developing aecidia, such a diploidization would be entirely without purpose. The position in a heterothallic Hymenomycete is different in that any part of the mycelium may subsequently give rise to fruit bodies, and must therefore contain nuclei of both compatibility groups; but in the rusts the aecidiospores arise from certain specialized groups of cells alone, and the formation of dikaryons throughout the whole of the thallus is thereby rendered unnecessary.

Reports of previous investigators. A final point to be considered in connexion with spermatial fertilization is the description, before the discovery of heterothallism in the rusts had been made, by various investigators of the origin of the dikaryophase farther back than in the aecidial fundaments. Such has been reported for a number of eu-forms by the following authors: Blackman and Fraser (27), Sharp (152), Tischler (160) Fromme (79), Kursanov (103, 104) and Lindfors (110). There is a possibility that the multinucleate condition of the mycelial cells which they describe was actually due to the presence of migrating nuclei on their way to the aecidial fundaments following natural spermatial fertilization.

Importance of spermogonia. It has been shown that in many micro-forms without spermogonia the dikaryophase is regularly initiated at some unknown point in the vegetative mycelium, but Buller has pointed out (37) that such forms, owing to the absence of spermogonia, are probably homothallic; his deduction has been subsequently confirmed by Ashworth (19) for *P. malvacearum*. It is certainly difficult to see how any rust which lacks spermogonia can exist in the heterothallic condition, as the spermatia are the only means by which fertilization of isolated monosporidial pustules can be brought about. In this connexion it is of interest to note that the aecidia of *P. alliorum*, according to Schroeter (151), are unaccompanied by spermogonia, while Dodge (68) has shown that the spermogonia of the

short-cycled rust *Gallowaya pinicola* are abortive and non-erumpent. Such rusts as these, were they once heterothallic, must have almost certainly subsequently gone over to the homothallic condition.

Homothallism. There are two ways in which homothallism may conceivably be achieved in the Uredineae: (a) by postponement of gametic differentiation until after the reduction divisions in the promycelium, or by mutation in the factors controlling it, with retention of the dikaryophase; and (b) by haploid parthenogenesis, the whole life cycle being passed in the haploid phase.

The former method appears to be not uncommon in certain Hymenomycetes, in which, after a length of time, previously self-sterile mycelia may spontaneously change over to a condition of self-fertility, as reported by Kniep (98), Vandendries (162) and Newton (123). Sass (149) attempts to explain homothallism in four-spored Hymenomycetes by assuming that gametic differentiation may occur, not during the meiotic divisions in the basidium, but at some point in the monospore mycelium; and cases of sporadic homothallism in a normally heterothallic fungus may possibly be accounted for on the same basis. Craigie (54, 55) has suggested the possibility of a spontaneous change from the haploid to the diploid condition in heterothallic rusts, but the great difficulty of ensuring complete isolation of monosporidial pustules makes experimental proof of this almost impossible. Allen (5) reports a case in *P. graminis* where two coalescent mycelia of the same syngamic group, which had remained sterile for several weeks, formed an abnormal sporophytic growth, which she interprets as an apparent abortive attempt at spore formation.

The latter method, that of haploid parthenogenesis, which has been demonstrated by Maire (115) and Bauch (24) for certain Hymenomycetes, appears to be not uncommon in the Uredineae. Olive (133) reported that the micro-form *Uromyces Rudbeckiae* is entirely uninucleate, and the same was found by the Moreaus (120, 121) for forms of *Endophyllum Euphorbiae* and *E. Kentranthi-rubri*, by Kursanov (101, 103, 104) for forms of *P. Pruni-spinosae* and *Aecidium leucospermum*, by Dodge (67) for forms of *Caeoma nitens*, by Brumfield, in unpublished work mentioned by Dodge (69) for a form of *P. Podophylli*, and by Cunningham (59) for *P. pulverulenta* and *Uromyces Selleriae*. The work of Dodge (67) is of particular interest, in that the uninucleate race of *Caeoma nitens* was shown to possess two-celled promycelia and to produce no spermogonia, although uninucleate forms with spermogonia were occasionally met with. Bauch (24) demonstrated that the uninucleate race of *Camarophyllus virgineus* produced two-spored basidia, in which no reduction division occurred; and it is almost certain that the two-celled character of the promycelia of the uninucleate non-spermogonial race of *Caeoma nitens* is correlated with the absence of a meiotic division. The lack of spermogonia may possibly be attributed

to loss of heterothallism consequent upon the adoption of an entirely haploid parthenogenetic life-cycle.

Explanation of genetical phenomena. It will be seen that the scheme according to which spermatial fertilization has been shown to take place in *P. phragmitis* can be made to render account for the phenomena noted by the investigators of rust genetics, namely, the occurrence of genetically different spores in the same hybrid aecidium cup, and the striking instances of 'maternal' cytoplasmic inheritance. In the first place, there is no reason why nuclei derived from different spermatia should not, in their wanderings through the fertilized thallus, be conducted along the hyphae of the vegetative mycelium into the same aecidial primordium, where a series of different dikaryons would hence be set up. Secondly, it is obvious from the cytological data presented that the spermatial nuclei, by the time they reach the basal cells of the fertilized aecidium, must retain practically none at all of their native cytoplasm, so that the protoplasm of the aecidiospores subsequently produced will be almost exclusively derived from the fertilized infection; thus the phenomenon of 'maternal' cytoplasmic inheritance finds an entirely convincing cytological explanation.

Nature of heterothallism. The question of the exact nature of heterothallism in the fungi has of late years received a good deal of attention. Since Blakeslee (28, 29) reported the existence of plus and minus strains in the Mucorineae, and assumed that in the heterogamic species the larger gametes were female and the smaller male, there has been a tendency in certain quarters to interpret the phenomenon of heterothallism purely in terms of sex. But not only has the assumption of 'male' and 'female' tendencies in the Mucors on the basis of gamete size been criticized by Cutting (60), but cases have been found in the higher fungi where heterothallism is obviously entirely distinct from sexuality. Gwynne-Vaughan and Williamson (85), for instance, have shown that although monospore mycelia of *Humaria granulata* fall into two distinct reproductive groups, all possess archicarps, antheridia being completely lacking. A similar state of affairs has been reported by Colson (47) for *Neurospora tetrasperma*. The conclusion to be drawn from these findings is that heterothallism in such fungi as these is to be interpreted, not in terms of sex, but in terms of self-sterility and cross-compatibility. This view is in the main held by Allen (2), Kniep (99), Cayley (41, 42), Prell (141), Ames (14, 15), Dodge (70, 71), Rice (145), Backus (22) and Drayton (75).

Spermatial fertilization. The phenomenon of spermatial fertilization in the rusts finds a close parallel in the diploidizing action of oidia demonstrated for *Coprinus lagopus* by Brodie (32, 33), and also in the fertilization by means of microspores shown to be possible in *Ascobolus stercorarius* by Dowding (73), in *Sclerotinia Gladioli* by Drayton (74, 75), and in *Neurospora tetrasperma* and *N. sitophila* by Dodge (71). Backus (22) has

also recently shown that in the parasitic Ascomycete *Coccomyces hiemalis* 'trichogynes' formed as extensions of the coiled archicarps grow out on to the surface of the leaf, where they are probably concerned with the reception of microconidia. This, together with the accounts of spermatial fertilization in the Lichens given by Stahl (154), Baur (23), and Bachmann (20, 21), provides a certain amount of data for purposes of comparison with the process of fertilization in the Uredineae.

Nature of spermogonia and spermatia. In the latter group the structure of the spermogonia is such as to suggest very strongly that they are male organs. If this is actually the case, we may infer that monosporidial infections of a heterothallic rust are in reality hermaphroditic self-sterile but cross-fertile haploid plants. That being so, sexual differentiation must have been manifested at a late stage in the growth of the thallus, namely, at the formation of the male spermogonia and the presumably female aecidial wefts. This conception, however, tends to break down when we consider the following facts:

(a) It has been shown that the dikaryophase may be initiated in compound infections by the intermingling and subsequent fusions of hyphae derived from different sporidia in the aecidial primordia. In the light of the foregoing hypothesis, these fusions must be regarded as taking place between purely female elements alone. Conversely, in the Hymenomycetes, the oidia of which are obviously comparable to the spermatia of the rusts, two mycelia of oidial origin may diploidize one another, as shown for *Coprinus lagopus* by Brodie (33); yet, according to the above theory, both these mycelia should be male. This inconsistency has already been pointed out by Dodge (71).

(b) Such a hypothesis as this demands the presence in the aecidial wefts of morphologically recognizable female organs as highly differentiated as, and equivalent to, the spermogonia. This demand, in spite of the description of 'egg cells' in the rusts by Andrus (16, 17), is hardly satisfied.

A more fitting solution to the problem seems to be arrived at if the terms 'male' and 'female' are provisionally entirely discarded, and the spermatia regarded as microspores having a syngamic function without any assumptions concerning sexuality. Although in the Uredineae they appear to have become specialized for a purely syngamic purpose, this is not the case in the homologous oidia of the Hymenomycetes, which can behave either as agametes or syngametes. The increasing importance in the life cycle of a fungus of one or other of these functions may well be associated with a corresponding specialization of the microspores in that direction. In the case of the heterothallic Uredineae, owing to the small chances of syngamically compatible sporidia settling sufficiently close to each other on the leaf to allow of direct internal diploidization, the spermatia are indispensable as a means of fertilization, and their specialization has

proceeded along lines similar to those followed by male gametes in organisms with full sexual reproduction. This would hence appear to provide an instance of independent evolutionary convergence.

VII. SUMMARY.

1. A complete account of previous work on the sexuality of the Uredineae is given.

2. *Puccinia phragmitis*, a hetereu-form with spermogonia and aecidia on species of *Rumex* and *Rheum*, and uredo- and teleuto-stages on *Phragmites communis*, was found by cultural experiments on *Rumex crispus* to be heterothallic.

3. Monosporidial pustules, if kept entirely free from insects, remained sterile, although numerous aecidial wefts were differentiated.

Monosporidial pustules fertilized by transferring spermogonial nectar developed normal open aecidia in all cases; the time required for this process was less in the case of older pustules than in that of younger pustules.

Over half of the bisporidial, and all of the polysporidial, infections protected from insects produced open aecidia.

4. In the polysporidial infections, the dikaryophase is initiated by the direct union, within the aecidial primordia, of hyphae derived from different sporidia. This union takes the form of the cell fusions described by Christman and other investigators.

5. In monosporidial infections kept strictly isolated, the central spermogonia, after functioning for some time, die, and are replaced by new spermogonia which develop centrifugally. The haploid aecidial wefts contain a certain number of multinucleate cells, and also show occasional cell fusions, which are, however, of a purely vegetative nature. With increasing age, the aecidial wefts and vegetative mycelium become highly vacuolate, except at the extreme periphery of the pustule.

6. In monosporidial infections fertilized by transfer of spermatial nectar, spermatia become attached laterally to the spermogonial periphyses by short bridging hyphae. Structures resembling small nuclei were seen in one case in both the spermatium and the periphyses with which it was fused.

Twenty-four hours after fertilization, numbers of very small nuclei of spermatial origin are seen in the vegetative mycelium, along the hyphae of which they migrate; partial dissolution of the septa occurs. At a later stage the spermatial nuclei enter the basal cells of the aecidial wefts, where dikaryons, consisting of spermatial and thallus nuclei in pairs, are formed. Fusion cells are occasionally seen at this stage, but there is strong evidence to show that they are derived from previous purely vegetative hyphal

fusions. Although multinucleate spore-chain mother-cells frequently occur, the spores to which they give rise are for the most part strictly binucleate.

In the fertilization of an old and vacuolate monosporidial infection, the protoplasm of the thallus cells through which the spermatial nuclei pass becomes regenerated. No new conductive hyphae are formed.

After the diploidization of the basal cells has been completed, a number of spermatial nuclei are left in the ground mycelium, but do not enter into conjugate relationship with the thallus nuclei. Their presence is probably due to the large quantity of spermatia transferred in experimental fertilization.

7. Syngamic reactions are manifested only between monospore mycelia which have reached a certain age; no fusions occur between germinating sporidia or between sporidia and spermatia.

8. An attempt to produce infection of the aecidial host by means of spermatia was unsuccessful.

9. A brief discussion of heterothallism and the nature of the spermogonia is appended.

In conclusion, the writer wishes to express his thanks to Professor F. von Wettstein for the facilities most generously placed at his disposal at the State Botanical Institute of Munich, where the experimental part of the work for this paper was carried out: to Professor V. H. Blackman for permission to conduct the microscopical part of the research at the Imperial College of Science and Technology, and also for much helpful criticism and advice; and to Mr. A. D. Cotton for the use of the library of the Royal Botanic Gardens Herbarium at Kew.

VIII. POSTSCRIPT.

Since this paper was sent to press five publications of considerable interest bearing on the question of heterothallism in the Uredineae have appeared:

- a. ALLEN, RUTH F.: A Cytological Study of Heterothallism in Flax Rust. Journ. Agric. Res., xlix, 765-91, 1934.
- b. ———: A Cytological Study of Heterothallism in *Puccinia sorghi*. Journ. Agric. Res., xlix, 1047-68, 1934.

The cytological phenomena connected with sporidial infection and spermatial fertilization in the heterothallic *Melampsora Lini* have been carefully investigated. The author comes to the conclusion that the transferred spermatia effect union with the fertilized thallus by growing in through the spermogonia, and perhaps also by entering through the epidermal cells of the host; in the latter case they apparently give rise to gametophytic hyphae which grow out, together with haploid hyphae

belonging to the fertilized pustule, to the epidermis, where cell fusions of the Christman type occur to form the sporogenous cells. The spermatial elements would hence appear to preserve their individuality right up to the base of the aecidial fundaments. In her study of heterothallism in *Puccinia Sorghi*, the author records a mode of development which in many ways shows a close parallel to that described for *P. phragmitis*. In unfertilized haploid pustules the central spermogonia die, and are replaced by new ones which develop centrifugally. The union of transferred spermatia with the periphyses of the fertilized infection, and the entry of spermatial nuclei by this means into the mycelium, is described. As in *P. phragmitis*, the spermatial nuclei of *P. sorghi* are at first smaller than those of the ground mycelium. A certain amount of evidence is also adduced for the existence, in naturally conrescent pustules belonging to syngamically compatible groups, of a direct process of diploidization by means of mycelial anastomoses, similar to that proved by the present writer for *P. phragmitis*. On the other hand, the interval elapsing between fertilization and production of aecidia in *P. Sorghi* is longer in older than in younger infections, and the central aecidial fundaments of an aged pustule lose entirely the capacity to form spores on fertilization, so that in cases of delayed fertilization the aecidia are formed only at the circumference of the pustule. The author construes the preponderance of multinucleate cells seen in the ground mycelium shortly after spermatial fertilization as evidence for a process of complete diploidization of the whole mycelium, vegetative and sporogenous alike. Might this appearance not be interpreted equally well, as in *P. phragmitis*, as being due to the presence of residual spermatial nuclei arising from the heavy experimental transfer of nectar?

c. ASHWORTH, DOROTHY: The Receptive Hyphae of the Rust Fungi. *Ann. Bot.*, xlix, 95-108, 1935.

d. —————: An Experimental and Cytological Study of the Life History of *Endophyllum Sempervivi*. *Trans. Brit. Mycol. Soc.*, xix, 240-58, 1935.

In the first of these papers it is demonstrated that emergent hyphae of the stomatal type and the intercellular type are of frequent occurrence in the rusts examined; their development is not confined to the aecidial stage, but may also accompany other spore forms. Rusts without aecidia and spermogonia may produce emergent hyphae. The presence of a thick cuticle on the host plant is detrimental to the development of intercellular emergent hyphae and may even suppress them altogether. In the second study, a cytological examination was made of the infection of *Sempervivum* by sporidia of *Endophyllum Sempervivi*. Spermogonia are the first organs to be developed, and occur markedly below stomata; emergent hyphae are frequently seen protruding through the stomata. The aecidial primordia are at first composed of uninucleate cells, and the diplophase

may be initiated by nuclear migration. Instructive experiments were made involving treatment of the spermogonia by burning out and covering with smears of various substances; the results obtained showed that the aecidia could complete their development without the intervention of the spermatia. The infections thus treated, however, were perhaps of mixed origin, containing mycelia of syngamically compatible groups; if this were the case, diploidization would probably occur within the leaf by the direct method as in the polysporidial infections of *Puccinia phragmitis*.

z. BURGESS, A.: Studies in the Genus *Uromycladium* (Uredineae). I. Proc. Linn. Soc. N. S. Wales, lix, 212-28, 1934.

The author presents a systematic and cytological study of the Australian genus of gall-forming rusts *Uromycladium*. Of particular interest are the 'intra-hymenial paraphyses' which are shown to be present in the mature spermogonia, extending in many cases to the ostiole. Binucleate examples of these paraphyses were found in old spermogonia, and it is suggested that they may possibly be concerned with the reception of spermatia.

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EXPLANATION OF PLATES IX AND X.

Illustrating Mr. Lamb's paper on 'The Initiation of the Dikaryophase in *Puccinia phragmitis* (Schum.) Körn.'

PLATE IX.

Fig. 1. Under surface of a leaf of *Rumex crispus* 12 days after inoculation with sporidia of *Puccinia phragmitis*, showing numerous monosporidial pustules with open aecidia; diploidization has occurred in some cases directly by coalescence, in others by the agency of insects. Natural size.

Fig. 2. Under surface of part of a leaf of *R. crispus* bearing an aged haploid unfertilized monosporidial pustule, photographed 28 days after inoculation by both reflected and transmitted light; the sterile aecidial wefts are seen as minute translucent areas. $\times 2$.

Fig. 3. Microphotograph of a section through an unfertilized aecidial fundament, 10 days after inoculation; the cells are predominantly uninucleate, being still in the haploid condition. $\times 440$.

Fig. 4. Microphotograph of a section through an unfertilized aecidial fundament, 22 days after inoculation; the cells have become swollen and extremely vacuolate, but in many of them the nucleus is seen as a darkly staining body attached to the cell wall. $\times 440$.

PLATE X.

(All figures were drawn $\times 1,000$, and reduced to three-quarters size in reproduction.)

Fig. 5. Haploid hypha in intercellular space, 72 hours after inoculation.

Fig. 6. A single spermatophore and three spermatia.

Fig. 7. Ostiolar periphyses of the bluntly pointed type in an unfertilized 12 days old monosporidial infection.

Fig. 8. An ostiolar periphysis of the bulbous type in an unfertilized 14 days old monosporidial infection.

Fig. 9-11. Cell fusions of the Christman type in the aecidial primordia of an 8 days old polysporidial infection.

Fig. 12-13. Vegetative cell fusions in the aecidial wefts of a 14 days old unfertilized monosporidial infection.

Fig. 14. Part of the tissue of an aecidial weft in a 22 days old unfertilized monosporidial infection; the cells have become greatly swollen and vacuolate, but retain their nuclei and a lining of cytoplasm.

Fig. 15. A hyphal cell of the vegetative mycelium in a 28 days old unfertilized monosporidial infection, showing extreme vacuolization.

Fig. 16. A spermatium united laterally with an ostiolar periphysis in an 11 days old monosporidial infection which was fertilized with spermatia 48 hours prior to fixation.

Fig. 17. A spermatium united with two adjacent ostiolar periphyses in an 11 days old monosporidial infection which was fertilized with spermatia 48 hours prior to fixation; the minute darkly staining bodies are probably spermatial nuclei.

Fig. 18. Part of a hypha of the vegetative mycelium in an 11 days old monosporidial infection which was fertilized with spermatia 48 hours prior to fixation, showing two thallus nuclei and three spermatial nuclei.

Fig. 19. Part of a hypha at the base of an aecidial primordium in an 11 days old monosporidial infection which was fertilized with spermatia 48 hours prior to fixation, showing spermatial and thallus nuclei and partially dissolved septa.

Fig. 20. Fusion cell of vegetative origin in an aecidial primordium of a 10 days old monosporidial infection which was fertilized with spermatia 26 hours prior to fixation; the spermatial nuclei have not yet reached this point.

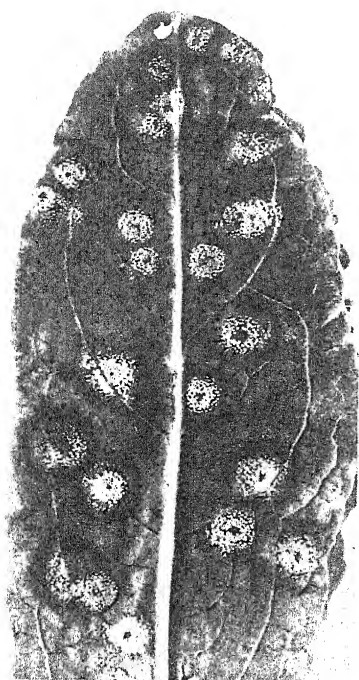
Fig. 21. Fusion cell, probably of vegetative origin, in an aecidial primordium of an 11 days old monosporidial infection which was fertilized with spermatia 48 hours prior to fixation; spermatial nuclei are present.

Fig. 22. Possible fusion cell of vegetative origin in an aecidial primordium of a 10 days old monosporidial infection which was fertilized with spermatia 26 hours prior to fixation; spermatial nuclei are present.

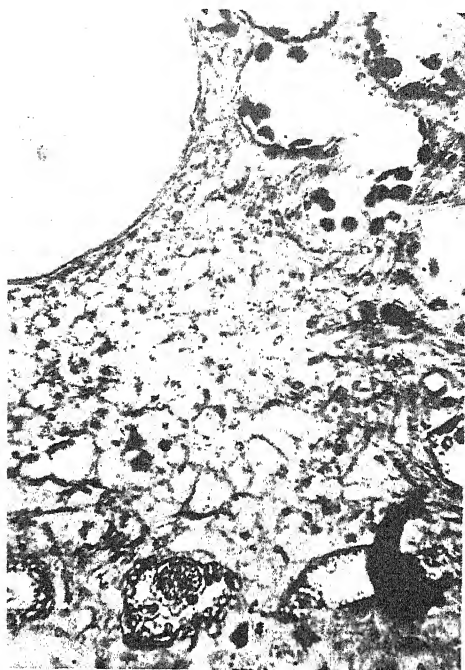
Fig. 23. Branched hypha in an aecidial primordium of an 11 days old monosporidial infection which was fertilized with spermatia 48 hours prior to fixation; four spermatial nuclei have entered from the ground mycelium.

Fig. 24. Conjugate division of two of the three nuclei in an aecidial basal cell of an 11 days old monosporidial infection which was fertilized with spermatia 48 hours prior to fixation.

Fig. 25. Part of the tissue of an aecidial primordium in a 20 days old monosporidial infection which was fertilized with spermatia 24 hours prior to fixation, showing diploid sporogenous cells and 'pathway cells' whose protoplasm has become regenerated through the influence of the spermatial nuclei. The 'pathway cells' contain, in addition to their own nuclei, residual spermatial nuclei.



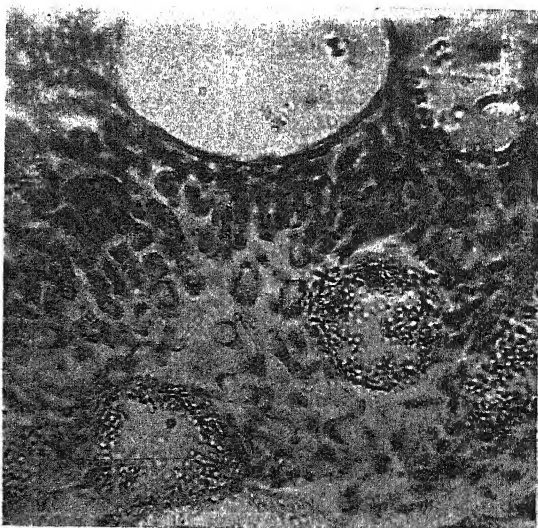
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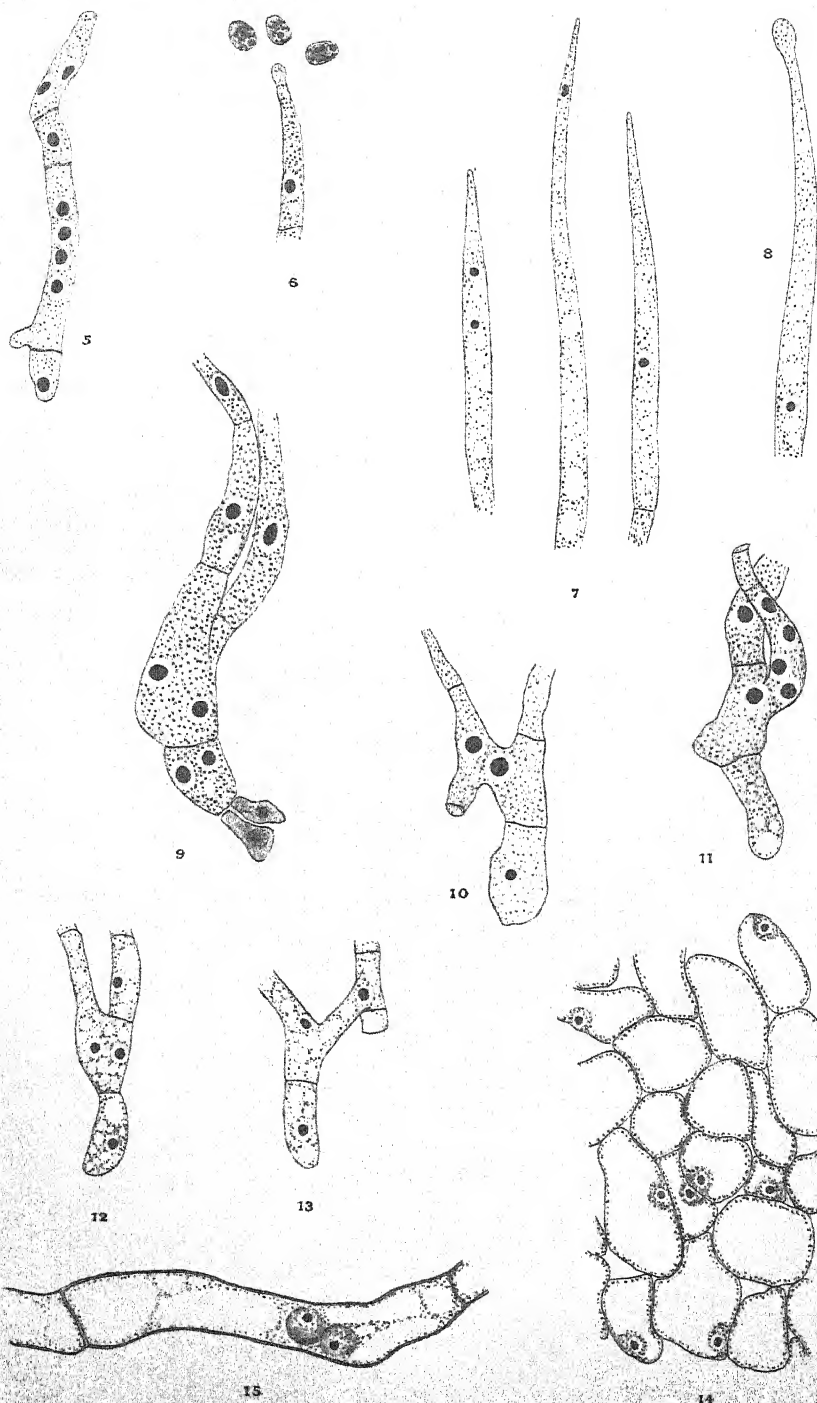
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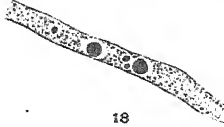




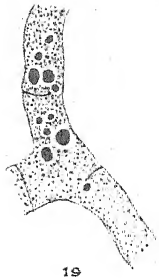
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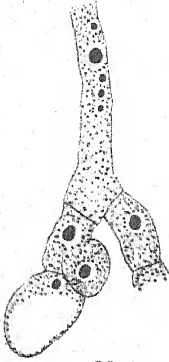
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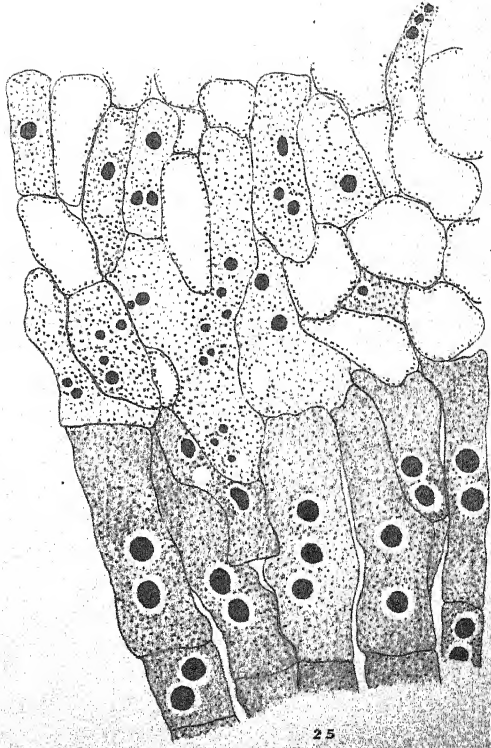
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The Life-history of *Rhodochorton violaceum* (Kütz.) comb. nov. (*Chantransia violacea* Kütz.).

BY

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(K. M. BAKER).

With eighteen Figures in the Text.

CONTENTS.

	PAGE
I. INTRODUCTION	439
II. MATERIAL	440
III. DESCRIPTION OF PLANT	
(a) Asexual plants	441
(b) Sexual plants	443
IV. DISCUSSION	448
V. SUMMARY	449
LITERATURE CITED	450

I. INTRODUCTION.

THE freshwater alga, which is the subject of this paper, has been most commonly included in the past in the genus *Chantransia*. This large Floridean genus has usually been considered as one comprising both marine and freshwater species. In a recent paper,¹ the writer (1) gave reasons for uniting the marine species (= *Acrochaetium* Näg.) with those of *Rhodochorton* Näg. to form the emended genus *Rhodochorton* (Näg.) Drew. Owing to the state of uncertainty existing with regard to many of the freshwater species, particularly with regard to their validity as species, their systematic position was not then discussed fully. As far as the species under consideration is concerned, these questions can now be answered, and the results of the following observations suggest that further work on nearly related forms is highly desirable.

Observations made throughout the growing season show that this alga,

¹ At the same time, it was pointed out that the generic name *Chantransia* should not be applied to such algae.

C. violacea (Kütz.) as it has been called, not only reproduces sexually in a manner characteristic of the simpler Florideae, but has a tetrasporic generation in addition. The most common method of reproduction, however, is by monospores. Thus the possibility that this alga is merely a stage in the life-history of any other Floridean species is ruled out and its independence is established. The presence of tetraspores suggests that it is a diplobiontic member of the Florideae.

The similarity of the species under consideration to some of the marine ones, such as *R. efflorescens* (J. Ag.) Drew, is so close that it seems undesirable to place it in a separate genus. It is therefore included in the genus *Rhodochorton*, as defined in the earlier paper above mentioned, and is subsequently referred to as *R. violaceum* (Kütz.) comb. nov. instead of *C. violacea* (Kütz.).

II. MATERIAL.

The material used for the observations, here recorded, has been collected from the upper stretch of the River Goyt, where it forms the boundary between Cheshire and Derbyshire.

Batrachospermum moniliforme, *Lemanea fluviatilis*, and '*Chantransia*' *chalybea* (Roth) Fries have also been found there. The protonemal growths of the first two have been seen with branches showing the metamorphosis into the adult form, but even without such branches, they are so different from *R. violaceum* that there is no possibility of confusion. '*Chantransia*' *chalybea* is green, and so is easily distinguishable from *R. violaceum*. Microscopically, also, these two species are quite distinct.

Collections have been made at various times since 1924, and during one growing season (1931-2) continuous observations were made.¹ Although *R. violaceum* has been found over a considerable length of the river, it shows a preference for the more turbulent parts, where the water is shallow and rushing over stones or small weirs. The first plants visible to the naked eye appear in late October, and through the following month there is a considerable increase in numbers, so that in December and January many of the stones are bright red owing to the richness of the growth. Only a few plants occur on *L. fluviatilis*, of which there are but young and rare specimens at that time of year. After January *R. violaceum* decreases in abundance (although it is always easy to collect material) until the second period of more rapid reproduction. Sometimes this starts in April, but may be delayed until May, probably depending on external conditions. This second period of growth is not as vigorous as the first and also differs

¹ The alga appears to have become rare during the 1933-4 season. The rainfall was exceptionally low during the summer and autumn of 1933 and the water of the river probably became considerably changed on account of work in connexion with the building of a reservoir. The decrease in abundance may have been due to one or possibly both of these factors.

from it in that the majority of the plants grow epiphytically on the *L. fluviatilis* and not on the stones of the river bed. After July the plants, which are confined to the stumps of the *Lemanea* filaments, are small and not obvious to the naked eye.

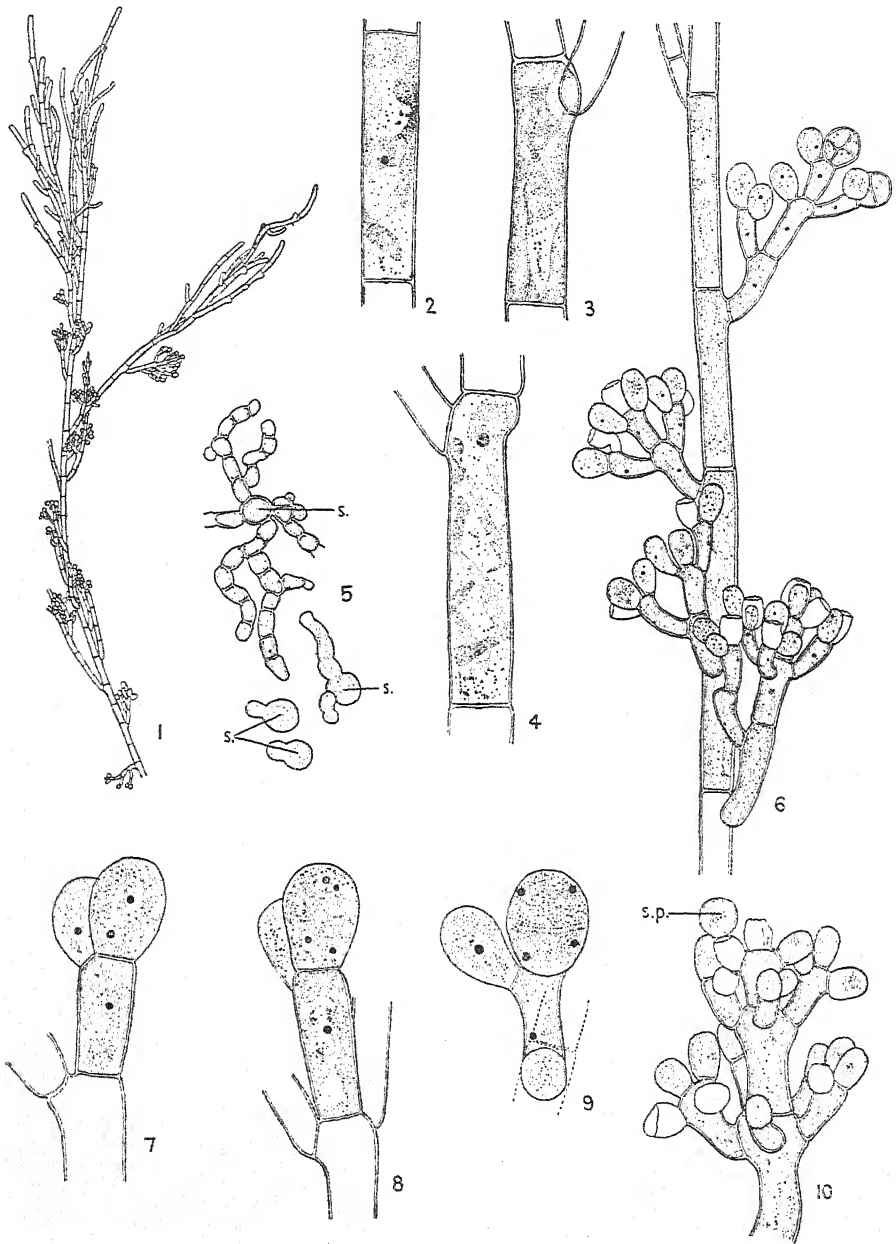
The plant is reproduced by monospores throughout the whole growing season, but sporangia appear to be much more abundant during some months than others. The branchlets bearing the monosporangia branch very richly during late February, March, and April, and so these plants have a very different appearance from that of plants of the earlier and later months. Tetrasporangia, which form among the monosporangia, are found in greatest numbers during the winter maximum. Occasional tetrasporangia have been seen during May and June as well, but never on sexual plants. Whereas the winter maximum seems concerned with asexual reproduction, the summer maximum, on the other hand, is associated with sexual activity. Carpogonia and cystocarps have been found during May and June only, and the development of antheridia is mostly confined to these months also, for only one male plant has been recorded for another date, viz. the end of July. It is of interest to note that cystocarpic material has been collected in late May from the River Wharfe, near Hubberholme, and antheridial material from a tributary of the same river, near Bolton Abbey, in late August.

Material used for detailed examination has been fixed in a mixture of 100 c.c. of 70 per cent. alcohol and 6 c.c. of 40 per cent. formaldehyde. Filaments have been stained whole in cotton blue in lactophenol except in cases where information regarding the nuclei was desired when Brazilin was used instead. The material, having been dehydrated and cleared, was mounted quite satisfactorily in Canada Balsam.

III. DESCRIPTION OF PLANT.

(a) *Asexual plants.*

On germination the spore gives rise to filaments which branch irregularly on the surface of stones or *Lemanea* (Fig. 5) and interweave to form a solid disc. Upright filaments arise from both the spore and cells of the filaments of the disc. These upright filaments branch abundantly, the branches having no definite arrangement and often equalling the main filaments in length. Before they are very long, branchlets of limited growth develop and on these the sporangia form (Fig. 1). The arrangement of the branchlets is usually irregular, but in early summer, plants with pairs of opposite branchlets may be found. Hairs terminate some of the branches and branchlets (Fig. 13), but they are never common enough to be considered a marked characteristic of the plant. The apical cells of the main filaments are slightly swollen at the tip and this is a noticeable



FIGS. 1-10. 1. Small part of plant, showing habit of growth and branchlets bearing monosporangia. $\times 87$. 2. Cell of main filament with very lobed plastid and prominent nucleolus. $\times 900$. 3. Similar cell with dense parietal plastid. $\times 900$. 4. Cell with ribbon-shaped plastids, spirally arranged. Nucleolus showing near apex of cell. $\times 900$. 5. Various stages of germination of spore on *Lemanea fluviatilis*. (*s.* = spore.) $\times 500$. 6. Filament with branchlets bearing both monosporangia and tetrasporangia. Note the formation of secondary sporangia inside the empty primary ones. $\times 500$. 7. Developing tetrasporangium containing two nuclei. $\times 1,400$.

feature of the plant. When fully grown, plants may measure up to 3 mm., those growing on stones tending to be larger than those on *Lemanea*.

The cells are cylindrical, and those of the main filaments average 9μ in diameter, but extremes of 6μ and 12μ have been measured. There is a greater variation in the length of the cells, but on an average it is 40μ . As would be expected, the cells of the branchlets are considerably smaller. Their average diameter is 6μ and length 18μ . Each cell contains a parietal plastid (Fig. 3), which is usually denser at the apical end of the cell and may not reach the basal end at all (Figs. 2 and 4). Sometimes the basal end of the plastid is ribbon-like (Fig. 2), and in other cells the whole plastid is dissected thus and the ribbons are spirally arranged (Fig. 4). No pyrenoid has been seen. The cells are uninucleate.

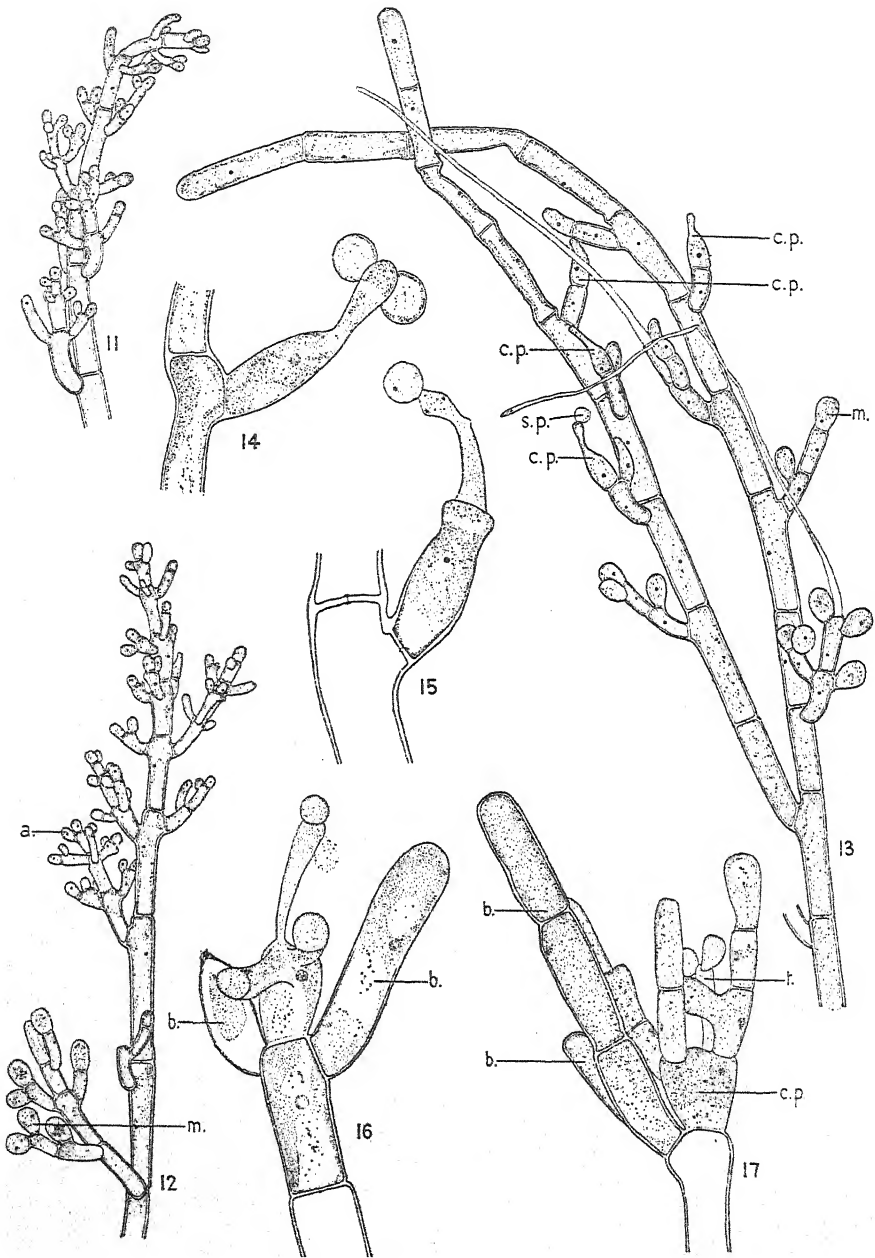
Monosporangia are formed in large numbers on the branchlets and eventually occupy all the terminal positions unless hairs are present (Figs. 1 and 6). In addition to the terminal sporangium, one or occasionally two lateral ones may be formed (Fig. 6). When mature, the sporangia measure from $5-8\mu$ in diameter and $8-10.5\mu$ in length, the average size being 7μ by 9.5μ . They are oval in shape, have a central plastid, and are uninucleate. The mature spore escapes through an opening at the apical end of the sporangium. A fresh sporangium may form inside the wall of an emptied sporangium (Fig. 6).

Although many plants bear monosporangia only, others bear tetrasporangia as well. They are never numerous and seem to be limited almost entirely to terminal positions in the branchlets (Fig. 6). The sporangia are broadly oval in section and contain dense plastids. When mature they average 9μ in diameter and 11.5μ in length and so are considerably larger than monosporangia. The first division of the protoplast is at right angles to the longer axis of the sporangium and usually starts before the second nuclear division. The second divisions are in planes more or less at right angles to each other. The mature spore is uninucleate. The development of the tetrasporangium is shown in Figs. 7, 8, and 9.

(b) Sexual plants.

R. violaceum is a dioecious species, but in exceptional cases an antheridial branchlet may develop on a carpogonial plant. Monosporangia may occur on the sexual plants (Fig. 12, *m.*), and when this happens they are limited to the lower part of the plant. As might be expected, they are much more numerous on the male than the female plants.

8. Four-nucleate tetrasporangium, showing the beginning of the transverse division of the protoplast. $\times 1,400$. 9. Slightly older tetrasporangium, the second protoplasmic divisions having begun. $\times 1,400$. 10. Antheridial branchlet with young, mature, and empty antheridia. An expelled spermium (*s.p.*) is seen near the apex. $\times 1,400$. The sporangia of Figs. 7, 8, and 9 are stained in Brazilin, all the other material figured in cotton blue.



FIGS. 11-17. 11. Apical portion of male filament. $\times 500$. 12. Filament bearing both antheridia (*a.*) and monosporangia (*m.*). $\times 500$. 13. Filament with carpogonia (*c.p.*) and also monosporangia (*m.*). Note hairs. (*s.p.* = spermatium.) $\times 500$. 14. Mature sessile carpogonium with two spermatia adhering to trichogyne. $\times 1,400$. 15. Fertilized carpogonium. Apical part of basal portion has begun to swell. Trichogyne beginning to decay. $\times 1,400$. 16. Two gonimoblastic filaments developing at apical end of basal part of carpogonium. One spermatium

Plants bearing sexual organs can be distinguished fairly easily from those bearing sporangia only. The male plants are characterized by their dense branching. From most cells of the main filaments arise two or more branchlets, which themselves are densely branched, each cell giving rise to two or more branchlets of a lower order. Owing to the difficulty of tracing more luxuriant growth, comparatively sparsely branched male filaments have been chosen for illustration in Figs. 11 and 12. The profusion of branchlets alone would make the antheridial plants easily distinguishable, but in addition, the cells of the main filament get progressively shorter and smaller towards the apex, the apical cell itself becoming an antheridium in many cases (Fig. 12).¹ Whereas the apical cell may be 3.5μ in diameter and only 6μ long, the third may be 4.5μ in diameter by 12μ , for example, the fifth 6.7μ by 22.5μ , and so on. Even the cells at the base of the plant tend to have a smaller diameter than those of the asexual plants. Owing to this progressive decrease in the size of the cells from the base to the apex of the main filaments, male plants are usually smaller than others.

The female plants are much less numerous than and very different in appearance from the male. Superficially they resemble sterile asexual plants, as the carpogonial branches, besides taking the place of branchlets, (Fig. 13, *c.p.*) are not numerous. However, owing to the cells of the filaments being shorter than those of the monosporangial plants, it is usually possible to distinguish them from sterile asexual plants even under a low magnification. The cells of the female plant average 6.7μ in diameter and 23.5μ in length, while corresponding ones of the asexual plants are 9μ in diameter and 40μ in length.

The antheridia occupy terminal and lateral positions on the cells of the branchlets (Fig. 10). They are formed in the same manner as monosporangia but in much greater numbers. Whereas it is usual for only one lateral monosporangium to develop in addition to the terminal one, two, three, or even more laterally placed antheridia are commonly formed (Fig. 10). A similar number may be formed laterally from any cells of the branchlet. In size the antheridium is considerably smaller than the monosporangium, the mature ones measuring $3-4\mu$ by $4.5-6\mu$. The antheridium contains an indefinitely defined body which takes up the colour of cotton blue in the same way as does the plastid of the vegetative cells, and so is presumably similar in nature. This is equally true of the

¹ A male plant, collected from a tributary of the River Wharfe in late August, differs from those of the River Goyt in that the cells of the main filament do not decrease in size and the branchlets are not opposite.

and the remains of a second adhering to trichogyne. Two branches (*b.*) have started to grow out from cell below carpogonium but one is dead. $\times 1,400$. 17. Later stage in the development of the cystocarp, showing three gonimoblastic filaments. The shrinking trichogyne (*t.*) with remains of two spermatia is seen behind. One of the two branches (*b.*), which have started to develop from the cell below the carpogonium, is almost completely hidden. $\times 1,400$.

spermatium, which is formed of the entire contents of the antheridium. When the spermatium is ready for liberation the antheridial wall ruptures in the apical region and the contents pass out. Even in fixed material, the expelled spermatium may sometimes be seen just outside the empty antheridium, and it then appears to be slightly larger than the latter (Fig. 10). This may be due to change in shape, as such spermatia are spherical and measure $3.7-5.5\mu$ in diameter. A liberated spermatium has a definite limiting layer, but it has not been possible to determine its nature. In no case has the formation of a secondary antheridium inside the empty walls of a primary one been seen.

The carpogonia usually terminate a one-celled branchlet (Fig. 13, *c.p.*), but sometimes the branchlet is two-celled or the carpogonium sessile (Figs. 14 and 15). In shape they are like those of the simpler Florideae (Fig. 14), and at the same time it should be emphasized that apart from their position, they are quite distinct from those cells with young, and therefore short, hair-like prolongations which are to be found at any time of year. The trichogyne is usually wider than such a hair and differs from it in that the apical region tends to be bulbous. In addition no wall separates the trichogyne from the basal part of the carpogonium until after fertilization, when empty or unused spermatia commonly adhere, leaving no doubt as to its nature (Fig. 15). The basal part of the carpogonium measures $4-6\mu$ by $9-13.5\mu$ and the trichogyne is usually slightly longer. The carpogonium contains a large plastid, which is less sharply defined than that of the vegetative cell. As the trichogyne begins to develop a small part of the plastid is carried forward into it. This eventually separates from that of the basal part of the carpogonium and occupies the apex of the trichogyne only (Fig. 14).

Nuclear fusion has not been followed, but after the adhesion of spermatia to the trichogyne, and presumably after fertilization, the contents of the basal part of the carpogonium separate from those of the trichogyne (Fig. 15). The trichogyne must disintegrate very quickly, for the examples figured are the only ones seen after the gonimoblastic filaments have begun to develop. The basal part of the carpogonium, on the other hand, enlarges, and in many cases is still obvious even when the cystocarp has reached maturity (Fig. 19, *c.p.*). The gonimoblastic filaments develop from the apical end of this portion of the carpogonium without any preparatory division and finally number four or five (Figs. 16, 17, and 18). They are usually unbranched and consist of a few cells (Figs. 17 and 18). One carposporangium is formed terminally, and each cell of the filament gives rise to one or two in lateral positions (Fig. 18). The carposporangia do not all mature together, those at the basal end of the first-formed filaments being mature when the terminal ones of the younger filaments are just beginning to form. They measure $6-7.5\mu$ by $7.5-10.5\mu$ and

contain a dense plastid. They are more broadly oval than the monosporangia and are often pear-shaped when mature. The carpospore escapes through a breaking of the wall at the apical end of the sporangium

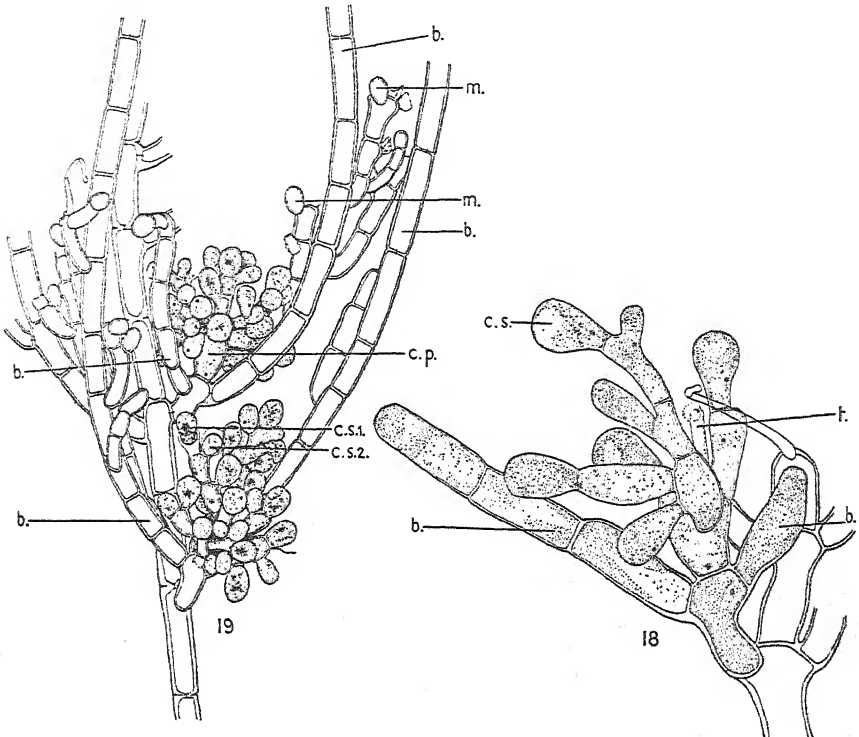


FIG. 18. Very young cystocarp, showing the development of the first carposporangia (c.s.). Shrivelled trichogyne (t.), with faint indications of spermatium, in background. Note the two branches (b.), which have originated from the cell below the carpogonium, one three-celled and the other one-celled. $\times 1,400$.

FIG. 19. Filament bearing two mature cystocarps. In the upper one, the basal part of the carpogonium (c.p.) is still visible. One carpospore (c.s. 1) of the lower cystocarp is being liberated and in the neighbouring empty carposporangium (c.s. 2) a second is forming. Monosporangia (m.) are being formed on the branches (b.) which develop below the cystocarp. $\times 500$.

(Fig. 19, c.s. 1). Occasionally a new carposporangium is formed inside the empty case of an earlier one (Fig. 19, c.s. 2), as in *R. rhypidandrum* (2) and *R. corymbiferum* (3).

In the case of stalked carpogonia, the cell below the carpogonium gives rise to two or sometimes three branches (Fig. 19, b.), which elongate considerably after fertilization. They are sometimes unbranched but, as in the example figured, may bear short branches with monosporangia (Fig. 19, m.). In two cases the cell immediately below the carpogonium has been seen to give rise to a small but typical monosporangial branchlet instead.

IV. DISCUSSION.

The preceding observations have established the fact that *R. violaceum* is undoubtedly an independent species. They also suggest that the life-history of this alga is that typical of the majority of the Florideae, as both tetrasporic and sexual plants have been found. Unlike the Florideae with this type of life-cycle, however, all plants can reproduce by monospores in addition, and hence the sexual and asexual plants can each reproduce themselves without the intervention of the other. In the nearly related *R. efflorescens* monosporangia are also found as well as tetrasporangia but, excepting rare instances, they develop on separate individuals. In the case of a species such as *R. violaceum*, where the individual plants appear to be short-lived and sexual reproduction is confined to a short period, monospores obviously play an important part in reproduction. Monospores alone could conceivably ensure the survival of the species under conditions unfavourable to sexual reproduction.

R. violaceum is a species showing a seasonal alternation of generations in the locality under observation, as sexual plants have been found in May and June only and tetrasporic plants are confined mainly to the winter months. A seasonal alternation is all the more striking in a species such as *R. violaceum*, as each generation can be repeated apparently indefinitely by means of monospores. The only influence the monospores appear to exert is in altering the relative numbers of tetrasporic, antheridial, and carpogonial plants expected. Tetrasporic are much more frequent than sexual plants, and male more frequent than female. The fact that monosporangia are most abundant on tetrasporic, less frequent on the male, and considerably less frequent on the female, is probably the main cause of this variation from the usual numbers.

It is evident from the account given that there are many points of resemblance between *R. violaceum* and some of the marine species of *Rhodochorton* (Näg.) Drew. Vegetatively, particularly in the shape of the chromatophores and the absence of pyrenoids, it resembles *R. efflorescens*. In addition, both species are characterized by an alternation of sexual and tetrasporic generations, the alternation being seasonal. They differ, however, in the details of sexual reproduction, and in this *R. violaceum* bears a closer resemblance to some of the other species of this genus. The antheridial branchlets are much more like those of *R. Macounii*, for example, and the carpogonia closely resemble those of *R. corymbiferum*, which is the only other species known to possess stalked carpogonia. The development of the cystocarp is essentially similar to that described for the genus as a whole, apart from the fact that the gonimoblastic filaments arise from the apical end of the carpogonium direct. In other species for which the cystocarp is known, the carpogonium divides by either one or

more transverse divisions to give a short filament, which afterwards branches. In view of the many other points of resemblance, this point of difference does not seem to be of sufficient importance to justify the separation of *R. violaceum* from the marine species.

As to the systematic position of the genus, some uncertainty is bound to exist until more is known about the various species, particularly with regard to cytological details. The extremely simple cystocarp suggests affinity with the Nemalionales, and the genus is indeed usually placed in this order. Svedelius (8) and Kylin (4), however, favour a limitation of the Nemalionales to haplobiontic forms, and there seems reason to doubt whether all species of *Rhodochorton* are such. It would seem that the emphasis in this case should remain on the structure of the carpogonium and cystocarp, and that the genus is best left, for the present at any rate, in the Nemalionales. Many investigators have placed the genus in the Helminthocladiaceae, but Kylin (2) created a special family for the marine species, and since he uses the generic name *Chantransia* he named the family the Chantransiaceae. There is much to be said in support of this separation, although the name is perhaps unfortunate since the generic name *Chantransia* was originally used for entirely different algae.

Reference should be made to two other filamentous freshwater Florideae, usually known as *C. Boweri* Murray and Barton and *Balbiania investiens* (Hassal) Sirodot, as sexual reproduction has been described for them. Apart from marked differences of the sexual organs, *C. Boweri* and *R. violaceum* are very alike. As, however, Murray and Barton (5) admit they could not follow the development of the cystocarp very fully owing to scarcity of material with sexual organs, and the figures appear somewhat inadequate, a further examination of material from the Kilpatrick Hills, the only locality on record for this species, seems highly desirable.

Although they differ considerably in general habit, there seems to be a close relationship through the manner of sexual reproduction between *R. violaceum* and *B. investiens* as described by Sirodot (7). Murray and Barton (5) have already suggested that the genus *Balbiania* is unnecessary, and there seem to be good reasons for including it in *Rhodochorton*. If this were done, a return would be made to the situation existing in the earlier days, when investigators, including Thuret (9) and Schmitz (6), classed the marine and freshwater species together in the one genus.

V. SUMMARY.

1. *R. violaceum* (Kütz.) comb. nov. has been observed throughout its season of growth, i.e. October to July in the locality studied. Sexual organs as well as tetrasporangia have been found in addition to monosporangia. The life-cycle is, in all probability, therefore, that typical of the diplobiontic Florideae. Since monosporangia develop on both sexual

and tetrasporic plants and other plants bear monosporangia only, the sexual and tetrasporic generations may be repeated independently of one another.

2. The carpogonia are extremely simple and are borne either on one- or two-celled branchlets or on the main filaments direct. The gonimoblastic filaments develop from the carpogonium itself. The antheridia are borne in great profusion on branchlets which usually arise in pairs. The male plants are small, the cells of the main filaments decreasing rapidly in size, from base to apex.

3. The alternation of generations appears to be seasonal, as tetrasporangia are confined almost entirely to the winter months and sexual reproduction to May and June only.

4. It is suggested that this species should be united with the allied marine species of the genus *Rhodochorton* (Näg.) Drew. In spite of the presumably diplobiontic nature of some species of the genus, it is left in a family of the Nemalionales for the present.

Before concluding, the author wishes to express her thanks to Professor W. H. Lang for his interest and help in connexion with this work, and also to the Council of the University of Manchester for laboratory facilities.

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December, 1934.

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On the Orientation of Stomata.¹

BY

G. E. SMITH.

With fifteen Figures in the Text.

INTRODUCTORY AND REVIEW OF DATA.

THE present investigation, which was undertaken at the suggestion of Professor E. J. Salisbury, has as its aim the elucidation of the factors determining the orientation of stomata, with special reference to those occurring on leaves. These factors may be connected with the morphology of the organ on which they occur, or with the physiology of its development.

For example, the parallel orientation which is so general a feature of the stomata occurring on most stems and petioles is also seen in many Monocotyledonous leaves, and might be held to be an indication of a common morphology, or of similar developmental conditions.

It is generally assumed that irregular stomatal orientation characterizes the leaves of Dicotyledons, and that Monocotyledons show regular orientation with the stomatal axes parallel to the long axis of the leaf, but it is well known that there are many exceptions to these generalizations.

Amongst Monocotyledonous types, *Arum maculatum* has a well-developed vein reticulum, and has stomata very irregularly orientated with reference to the leaf as a whole. In *Paris quadrifolia*, where again the venation is of the reticulate type, but less pronounced than in *Arum*, the stomata are irregularly orientated over most of the leaf surface, and regularity is approached only where the main veins converge at the base of the leaf, and where the venation is approximately parallel. Similar tendencies are shown to a less extent at the apex of the leaf. *Tamus communis* also shows irregular orientation coupled with a reticulate venation.

Among Dicotyledons it is usual to find the stomatal axes exhibiting a great diversity of orientation upon the same leaf, but it is possible that there is a higher proportion of species with a fairly regular orientation than is usually imagined, further, it would seem probable in many instances that where the stomata at first appear to be quite irregularly orientated in reference to the leaf as a whole, a careful analytical examination might

¹ Part of Thesis approved for the Degree of Master of Science in the University of London.

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show some degree of correlation between stomatal orientation and leaf structure.

Dicotyledons do not invariably show irregular orientation of stomata, although exceptions are comparatively rare. Those Dicotyledons which have a regular stomatal orientation can be divided into two categories, firstly, those with a regular arrangement as well as a regular orientation with parallel stomatal axes; and, secondly, those which show parallel orientation without a regular arrangement. The word arrangement is used merely to imply the disposition of the stomata on the lamina with regard to each other, and without consideration of the direction of their long axes.

If the development of the leaf follows a simple course from the meristematic stage, and the primary arrangement of the epidermal 'mother-cells' is not disturbed by differential growth, both a regular *arrangement* and *orientation* of the stomata would be expected if the stomatal initials are formed from the epidermal mother-cells in a regular way.

In all observed instances of regular stomatal *arrangement* there is also regular *orientation*, but often when the orientation is regular the arrangement is not. Stomatal orientation may be completely parallel even where the outline of the epidermal cells is so extremely sinuous that any linear arrangement, which may have obtained at an earlier stage of development, seems entirely lost.

It thus seems that there must be other factors, in addition to the regular divisions of the primary meristem, which play a part in the determination of stomatal orientation, although such regular divisions may sometimes provide an adequate explanation of observed facts. (Fig. 1.)

The following is a brief résumé of data already recorded on the subject of stomatal orientation, which has largely been abstracted from various anatomical works, but in addition a large number of species have been examined by the present writer. A summary of the data will be found in tabular form on pp. 474 and 475 (Table VI A and B).

In the Ranunculaceae a variety of types of stomatal orientation occur, and an interesting series is shown in various species of *Ranunculus*.

R. graminifolius, amongst other xeromorphic characters, has nearly rectangular epidermal cells, and the stomata (mostly on the inner surface of the rolled leaf) are almost Monocotyledonous in the regularity of their orientation, while the epidermal cells show an arrangement in longitudinal rows.

In *R. lingua* the outline of the upper epidermal cells is very wavy, but the arrangement in rows is not lost; in the lower epidermis, where the undulations are more marked, the linear arrangement is not so plain.

R. flammula shows still less regularity in the arrangement of the epidermal cells, but in this species, as well as in *R. lingua*, the stomata are all very nearly parallel to the long axis of the leaf. These Ranunculaceous

leaves differ from those of typical Monocotyledons in the occasional occurrence of a stoma at a considerable angle to the long axis of the leaf.

The stomatal orientation on the segments of divided leaves of *R. aquatilis* (agg.) when growing on dry mud, is regular and parallel to the long axis of the segment. A similar condition obtains in *Nigella*.

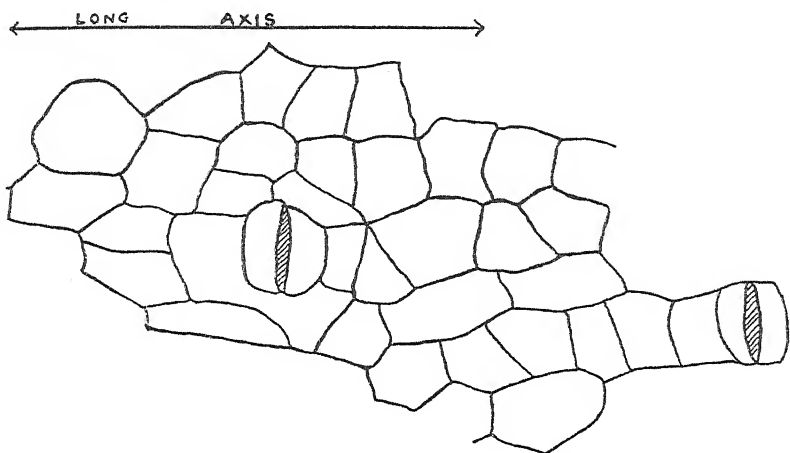


FIG. 1. *Tamarix*. Surface section of scale-leaf.

The lobed leaves of *R. sceleratus*, of *Aconitum napellus* and of *Paeonia* sp. show stomatal orientation parallel with the midrib of the lobes, and irregular tendencies are shown only where one segment joins another.

Ficaria verna will be described in detail later—the leaf is three lobed, with veins radiating into the lobes from the petiolar attachment. The orientation follows very closely the direction of the main veins for each region. Similar observations have been made on the upper surface of the leaf of *Nymphaea lutea*. The seedling leaves of *Caltha palustris* strongly resemble those of *Ficaria verna* in the mature condition, and the stomatal orientation appears very similar in the two, but mature leaves of *Caltha* show a more complex venation, and the stomatal orientation is less regular.

It may here be remarked that Salisbury (15) has shown for *Ranunculus parviflorus* that stomatal orientation is determined at an early stage; microtome sections of the young epidermis show that the same proportion of irregularly orientated stomata occurs in the immature as in the adult leaf.

In the Leguminosae, while the stomatal orientation is irregular in the normal, broad-leaved forms, a number of exceptions are met among those species with narrow pinnules and phyllodes. Here the stomata most often have their long axes parallel to the long axis of the organ on which they occur (*Viminaria denudata*, *Daviesia divaricata*), but they may be orientated transversely (*Carmichaelia australis*, *Alhagi*, and others).

In *Carmichaelia australis*, transverse orientation occurs on the leaflets of the trifoliate leaves, as well as on the flattened stem.

On the stipules of *Vicia faba* the stomatal orientation is parallel to the long axis of the organ, and the same condition occurs in the first few leaves of the seedling, which are almost entirely stipular in character. Tyler (19).

The Saxifragaceae are characterized by a distribution of the stomata in patches, alternating with stomate-free areas composed of large-sized epidermal cells.

The stomata of *Chrysosplenium oppositifolium* show a distinct tendency towards a uniform orientation in any given patch, which tendency is not so marked in *C. alternifolium*.

It may be remarked in passing, that this distribution of stomata in patches may for several reasons be regarded as an expression of water economy. Something similar is seen in the Apocynaceae: *Plumeria* has stomata evenly distributed in the areas marked out by the vertically transcurrent veins, while *Strophanthus* shows a tendency towards grouping of the stomata, and in *Nerium oleander* the stomata occur in pits.

In such instances the orientation of the stomata appears to be to some extent affected by their arrangement and distribution. In the small family of the Bruniaceae, most species have stomata with their long axes parallel with the midrib. From the description given by Solereder (17, vol. i, p. 334) it would seem that here the orientation may be due to regular development, since the stomata occur in longitudinal rows on either side of the midrib (1, 2, or 4-5 rows). In *Staavia radiata* the pore is at right angles to the median vein.

A high proportion of species in the Proteaceae have parallel venation and stomata with their long axes parallel with the long axis of the leaf (*Leucadendron*, Fig. 2).

In the Cactaceae the direction of the pore is a characteristic feature of certain genera. Vöchting (22) states that in *Pfeffera* and *Rhipsalis* the pore is at right angles to the long axis of the shoot, and Lauterbach (10) states the same for *Echinopsis* and *Cereus*. In *Lepismium* (Vöchting) and in *Epiphyllum* and *Opuntia* (Lauterbach) the pore is parallel with the long axis of the shoot, while in the other genera it is quite irregularly orientated.

Amongst the Chenopodiaceae transverse stomatal orientation is common both on leaves and on stems (Table VI B). Many of the species which show transverse orientation have centric leaves (*Suaeda*, *Salsola*).

Batis maritima, a monotypic species growing on the sea coast of the Sandwich Islands, has opposite, fleshy, linear leaves (Willis, 24) with stomata mostly orientated at right angles to the axis of the leaf. The stomata are placed transversely also on the stem (Solereder, 17, vol. ii, 669 and 1032).

Tamarix, another sea-coast shrub, has transversely orientated stomata

on the scale-like leaves and on the green parts of the stems and branches (Fig. 1).

Other species exhibiting transverse orientation of the stomata are found in various families of parasitic and semi-parasitic plants.

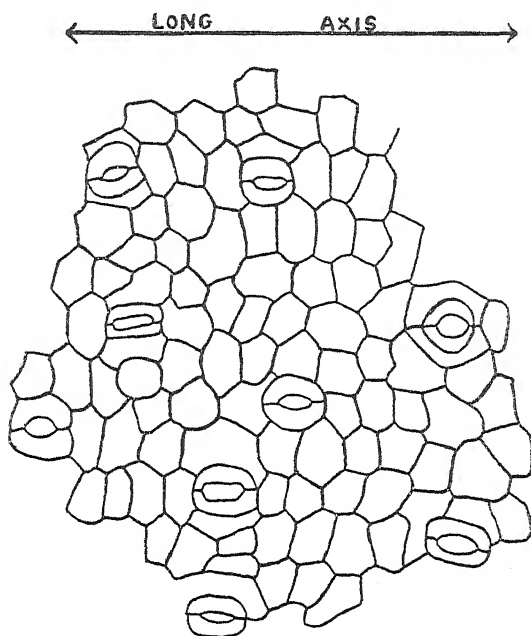


FIG. 2. *Leucadendron*. Surface section of lower epidermis.

All species of *Cassytha* have transversely orientated stomata on the leaves (when present) and on the stems; commonly they are arranged in rows. Chatin (8).

In the Santalaceae, *Thesium humifusum* has stomata at right angles to the long axis of the leaf, and here they are arranged in rows. On the stem they are transverse, occasional ones are seen to be oblique, and a rather higher proportion are oblique at the nodes.

In the Loranthaceae, *Viscum* has stomata at right angles to the long axis of the stem, but with their long axes pointing in all directions on the leaf. Chatin (8), Van Tieghem (18).

In *Arceuthobium americanum* and a number of other species of the Loranthaceae, the stomata are transverse on the stem.

As was mentioned on p. 453, transverse orientation of stomata occurs widely amongst some of the xeromorphic Leguminosae (*Carmichaelia*, *Alhagi*; Australia, *Anarthrophyllum*; Andes, *Krameria triandra*; Mexico and Chile).

It has been said that the parasitic habit at its inception was primarily important for a supply of water, and that nutritional parasitism is probably of secondary origin. The only three groups of plants in which transverse orientation of the stomata commonly occurs, are halophytes, desert plants, and plants of parasitic or semi-parasitic habit.

It is, therefore, possible that transverse stomatal orientation may be in some way an expression of xeromorphy, since the obvious common factor for the habitats mentioned is that the water supply may be deficient. It has to be remembered, however, that although most known examples of transverse orientation are to be found in these groups, all other types also occur in them, and all that is suggested is that a transverse orientation of the stomata may be one of the many reactions of plants to dry conditions. At the time of writing, a species of *Rhipsalis* (with transverse orientation) is being grown in a saturated atmosphere, and a control in a normal atmosphere.

The alternative possibility, which is less easy of verification, is that stomatal orientation may be an hereditarily determined character and in no way affected by the environment.

In addition to the two kinds of parallel orientation described above, a number of other instances of regular orientation have been observed, and may be briefly mentioned.

In *Hydrocotyle* the stomata were observed to be regularly orientated where the epidermal cells were elongated over the veins, but otherwise were rather irregular.

In *Pteridium aquilinum* it was found that the stomatal axes followed the direction of the veins very closely, marked irregularity only being seen near vein junctions. *Mengyanthes* showed a somewhat similar type of stomatal orientation.

Euphorbia porlandica, *Astragalus danica*, *Corrigiola littoralis* each showed a large proportion of stomata parallel to the long axis of the leaf, but with an appreciable number markedly divergent.

Melampyrum pratense has elongated leaves, the main laterals and the midrib of which have a pronounced effect upon the shape of the epidermal cells above them. The stomata situated near these regions nearly all lie with their long axes parallel with the direction of the veins. Irregularly orientated stomata occur frequently near the middle of the lamina, but near the base the stomatal orientation is parallel, even though the epidermal cells are very sinuous in outline. In the young leaf from the stem apex, the epidermal cells are smaller but already sinuous, and there appears to be a higher proportion of parallel orientated stomata, particularly at the base and tip of the leaf.

In *Trientalis europea* the midrib and main laterals form, on the under-side of the leaf, a prominent raised network of ridges above the general

leaf surface, and so divide the area into a number of nearly rectangular areas. These areas, particularly those just inside the peripheral vein, show a high proportion of stomata parallel with the sides of the rectangle. (Fig. 3, A and B.)

A much lower correlation is seen in those areas which are nearer the middle of the leaf, while few stomata have their long axes parallel with the midrib itself. In *Lysimachia vulgaris* (grown in cultivation), when the leaves are young, many stomata are parallel with the lateral veins. This plant is of considerable interest, as the lower leaves on the stem are simple and scale-like, and, passing upwards, successive leaves show a more and more complicated structure, until those near the flowering branches have a well-developed vein-reticulum. The stomatal orientation on the lower scales is parallel with the simple longitudinally running veins. The orientation is very irregular on the higher adult leaves, and a gradual transition between the two types is seen on the leaves of the intermediate part of the stem.

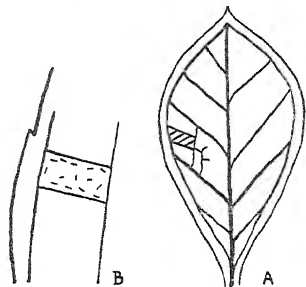


FIG. 3.

In *Buxus* the stomata near the midrib are mostly orientated parallel with it, while those nearer the periphery are orientated parallel with the lateral veins. Cross veins are not strongly developed. (Figs. 4 and 5.)

In this review of types of stomatal orientation only those species with at least some regularity of orientation have been mentioned, but in the course of the work a large number of examples have been met, of the irregular orientation which is considered to be typical of Dicotyledons.

The following few examples may be mentioned: *Parthenocissus quinifolia* (but regularity is seen on stipules and the tips of young leaves), *Hippocrepis*, *Alchemilla alpina*, *Astragalus glycyphyllos*, *Sambucus nigra*, *Sonchus palustris*, *Ficus* spp., *Tilia europea*, *Betula*, *Fagus sylvaticus*, and *Fagopyrum*. The epigeal cotyledons of the two last-named species show completely parallel stomata.

The presence of stomata on floral leaves, coupled with their simple venation, makes certain observations of interest. Thus the orientation of the stomata on the perianth leaves of *Paris quadrifolia* was completely regular in the specimen examined.

From an examination of the figures in Muller's 'Anatomie der Blumenblätter', it would seem that parallel stomatal orientation is of common occurrence on petals and perianth members.

Sambucus nigra.

The fact that an evident correlation existed between the orientation of the stomata and the direction of the veins in so many instances, suggested

that an analytical examination of a leaf where no such obvious relationship obtained might prove of value. *Sambucus nigra* was chosen, and both sun and shade leaves were collected.

Preliminary observations were made on strips from the lower epidermis of shade leaves. The position of the veins of an area was drawn approxi-

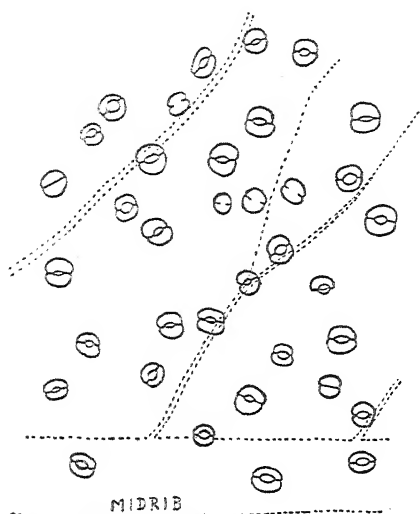


FIG. 4.

FIG. 4. *Buxus*. Young leaf. Stomata and veins near midrib.

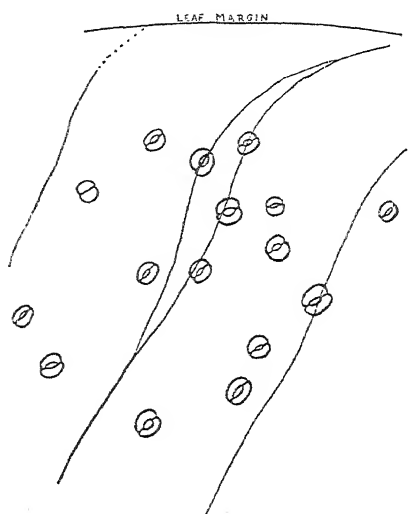


FIG. 5.

FIG. 5. *Buxus*. Young leaf. Stomata and veins at edge of lamina.

mately from the elongation of the epidermal cells above them, and then the stomata present in the same area were classified into four divisions, namely, (*a*) parallel to the nearest vein, (*b*) at right angles to the nearest vein, (*c*) and (*d*) oblique in either direction to the vein. By such grouping, although the results were often rather indefinite, it was found that usually the number falling into the first category (viz. parallel to the vein) was higher than the number in the other categories. The above method is not very reliable, and for all later determinations a camera lucida was used, and the orientation shown by drawing a line through the long axis of the stoma. The exact position of the stoma was recorded by drawing the outline of one of the guard cells. (Figs. 6 and 9.)

A complete strip obtained from the region between two lateral veins was examined in this way in nine areas (Fig. 5A). An undoubted correlation was found to exist between the direction of the stomatal pore and the direction of the smaller veins, but not with the main laterals which arise directly from the midrib. A possible explanation of this observation will be seen later when the development of young leaves is discussed.

Since the course of the smaller veins, and particularly of the vein endings, is not indicated by the shape of the epidermal cells above them, it is not possible to take account of the whole vascular system when epidermal strips are employed.

The most satisfactory of several methods tried was to clear the entire leaf in Eau de Javelle, and after careful washing to mount it whole. Some mounts were made in weak saffranin in dilute glycerine; the veins became well stained after some time, and often the stomata were shown up clearly, while the rest of the leaf was comparatively unstained.

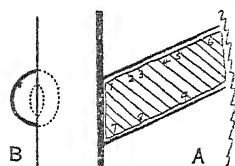


FIG. 6.

An alternative method was to stain in bulk in ammoniacal basic fuchsin by the methods employed by Barratt and by Bond (3) and (6).

When this procedure was followed the entire leaves were mounted in euparal between thin slides. From these preparations a series of drawings was made—the vascular tissue was first drawn, and then by altering the focus the stomata present in the same area were drawn as indicated previously.

Copies of two such drawings are shown for a mature shade-leaf in Fig. 7 A and B, and for a mature sun-leaf in Fig. 8.

Although some stomata have no evident relation to the veins, yet many show in a striking way how the orientation follows the direction of the veins. The higher frequency on sun-leaves makes it possible to find regions sufficiently near to a vein and yet including enough stomata to admit of numerical treatment, and below are given the results of two determinations.

TABLE I.

A. 27 stomata.

Angles between long axes of stomata and direction of vein.

Grouping	0°-20°	20°-40°	40°-60°	60°-80°	80°-90°
Number of stomata	11	6	3	5	2
Percentage of total	40.7	22.0	11.0	18.0	7.4

B. 26 stomata.

Angles between long axes of stomata and direction of vein.

Grouping	0°-20°	20°-40°	40°-60°	60°-80°	80°-90°
Number of stomata	12	5	4	1	4
Percentage of total	46.0	19.2	15.3	3.8	15.3

In control determinations made in relation to arbitrary axes drawn on the paper, vein-free areas were used, and since the number of stomata in a single area was not sufficient for the purpose, the stomata from a number of

such patches were superposed on the paper by moving the slide laterally by a mechanical stage, so that fresh areas became available for drawing under the camera lucida. When the results were grouped as before, the

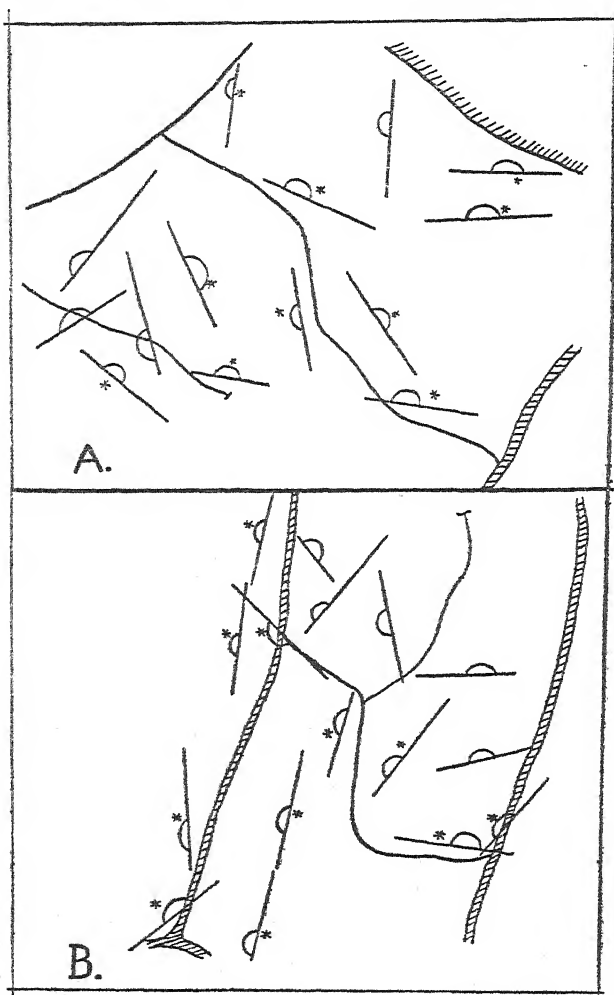


FIG. 7 A and B. *Sambucus nigra*. Stomata and veins of a shade-leaf. Stomata marked * show a correlation between their orientation and the vein direction.

distribution was much more nearly what would be expected on chance, though the numbers were such as to make sampling errors appreciable.

The results are summarized in Table II.

A comparison of the numbers in Tables I and II very strongly supports the conclusion previously stated on p. 457, viz. that there is in *Sambucus nigra* a correlation between the direction of the long axes of the stomata and the direction of the underlying vascular tissue.

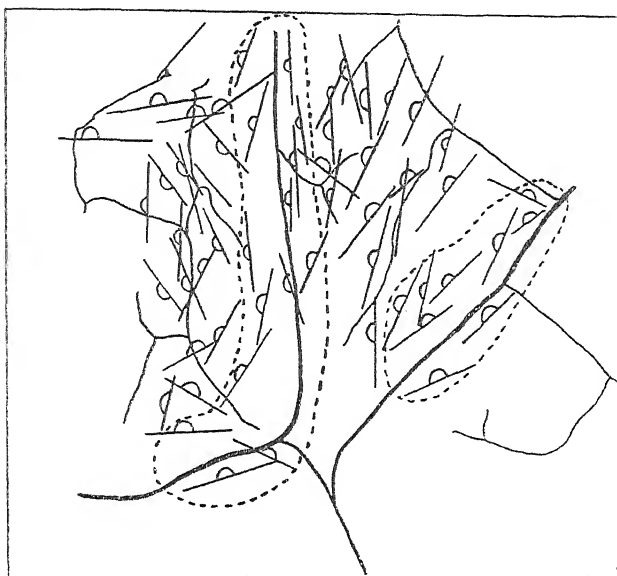


FIG. 8. *S. nigra*. Stomata and veins of a sun-leaf. Correlation in areas marked out by dotted lines.

TABLE II.

	Angle groups	0°-20°	20°-40°	40°-60°	60°-80°	80°-90°
Arbitrary axis No. 1	(Number of stomata) (Percent. of total (21))	5 23·8 %	3 14·3 %	3 14·3 %	3 14·3 %	7 33·3 %
Arbitrary axes Nos. (2 + 3)	(Number of stomata) (Percent. of total (25))	6 24 %	6 24 %	6 24 %	4 16 %	3 12 %
Arbitrary axis No. 4	(Number of stomata) (Percent. of total (24))	5 20·8 %	5 20·8 %	3 12·5 %	7 29·2 %	4 16·6 %
Total no. of stomata (70)		16	14	12	14	14
Average percentages		22·8 %	20 %	17·2 %	20 %	20 %

Consideration of the above results suggested that a study of developmental stages might possibly shed some light on the variations of orientation in the adult. Leaves of *Sambucus* dissected from the unopened bud had no stomata and no recognizable veins except traces of the midrib initial. Leaves obtained from a stool-shoot showed all stages of development, and developing stomata were found on those which were scarcely open, and the leaflets of which had to be unrolled. Transparencies of these leaves were prepared and a series of leaflets mounted.

The stomata which had been differentiated near the apex of the terminal leaflet were almost without exception parallel to the long axis of the leaflet (as also were the water stomata). No lateral veins were developed at the apex itself, though lateral vein initials consisting of elongated, but

otherwise undifferentiated cells could be seen a little nearer the base (Fig. 9). The size of the leaf teeth in these young leaves is great in proportion to the

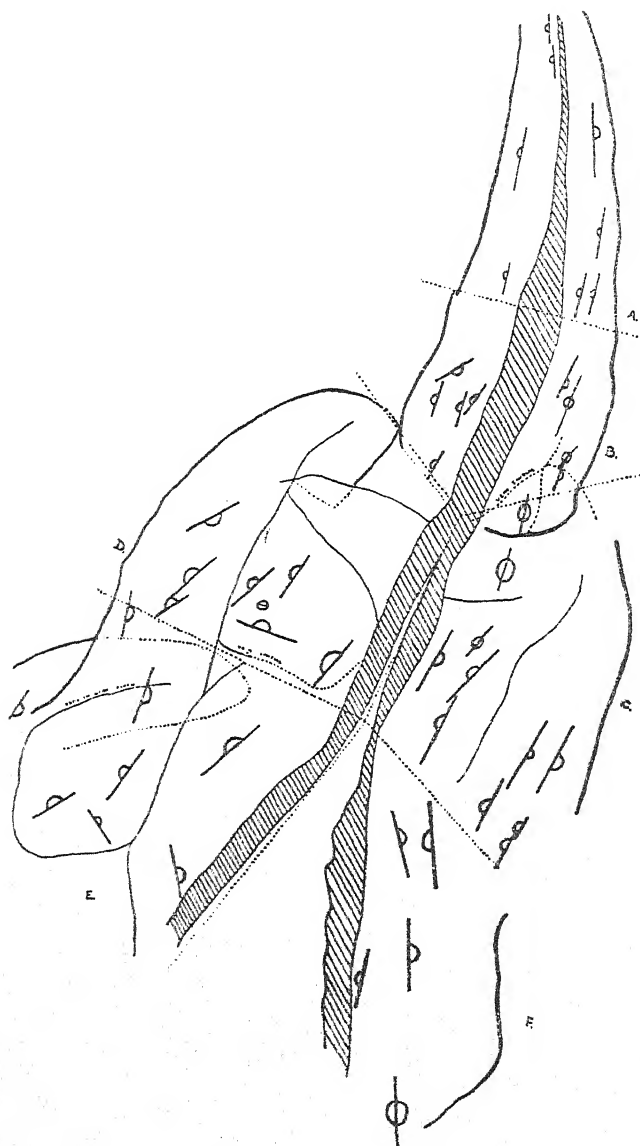


FIG. 9. *S. nigra*. Apex of very young leaf showing high correlation between the stomatal orientation and the midrib (shaded). Other veins scarcely differentiated. (Leaf partly split for mounting.)

area of the laminar region, which later expands rapidly. This high correlation is shown on the young leaflets all down the rachis, and, where

there are actively developing stomata, it is usually those that are least mature which show the lowest correlation with the direction of the already existing veins. Since these are the stomata which are being developed later than the adjacent vein, it may be concluded that the correlation between vein direction and stomatal orientation, is highest when the stomata and the veins are differentiated simultaneously.

This would seem to indicate that the veins during their differentiation may exercise a directional effect on the orientation of the stomata.

It should be mentioned that the development of the leaflets on the rachis is basipetal in *Sambucus*; further, the apex of any given leaflet matures before other parts of the same lamina.

The results obtained from young and old leaves of *Sambucus* are discussed on p. 475.

Ficaria verna.

It was thought desirable to repeat these methods of investigation on a simple, entire leaf, and for this purpose that of *Ficaria verna* was chosen.

The leaf has an approximately radial type of construction, and, broadly speaking, is composed of one forward lobe and two backward lobes. The main veins radiate from the petiole into the three lobes. On a first examination of the mature leaf it was seen that there was a high proportion of parallel stomatal orientation, which as one passes round the leaf from A to B (Fig. 10), changes so as always to be parallel with the main radial veins nearest a given area.

Young plants were taken and the leaves dissected out from the leaf-sheath, still tightly rolled, and it was found that quite small leaves possessed stomatal initials. Stomata were present on the leaf sheaths, and were all parallel to the long axis of the sheath.

Young leaves cleared in Eau de Javelle were stained with ammoniacal fuchsin and light green. One immature leaf was studied in detail in the five sample patches shown in the plan of the leaf (Fig. 10).

The lower epidermis was used throughout. The stomatal orientation is similar in the upper epidermis, but the frequency is lower.

For each sample region selected the vascular system of that area was drawn, and then the stomata were drawn on the same paper—as shown in Fig. 11, which represents area No. 5 (Fig. 10). The angle made by each stoma with the nearest radial vein was measured and tabulated. In position (2) there was no radial vein, but the general resultant direction of the veins present was radial, and the angles were measured with this as a standard. The results are shown graphically in Fig. 12 and in Table IV.

A feature of interest which came out of this investigation was that one lobe of the leaf was more mature than the other. This is strikingly shown

when the proportion $\frac{\text{stomatal initials}}{\text{stomata with cross walls}}$ is calculated for the various sample areas. The leaf is rolled spirally in the bud, and the outer parts develop earliest.

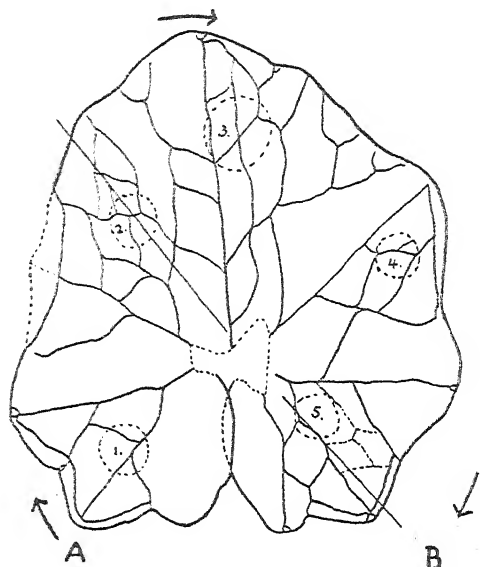


FIG. 10. *Ficaria verna*. Plan of leaf.

TABLE III. (See Fig. 10.)

Position.	Percentage $\left(\frac{\text{initials}}{\text{initials} + \text{stomata}} \right)$
1	43 % (nearly)
2	40.5 %
3	29 %
4	27 %
5	20 %

It is difficult to estimate the degree of immaturity of a leaf during its development, and it is suggested that the ratio initials (stomata + initials) might be of use in comparing the stages of development reached either in different parts of the same leaf, or in comparable parts of different leaves. This would be valueless in those instances where stomata continue to be differentiated throughout the life of the leaf.

In view of the results obtained it was clearly desirable to discover how far the high correlation observed in the young leaf was preserved when the vein network had fully developed, and when the corresponding stomata had differentiated in the adult leaf.

The leaf selected was examined in detail in five sample areas in a

similar way to that described for the young leaf. It was found that the correlation between the direction of the stomatal axes and that of the

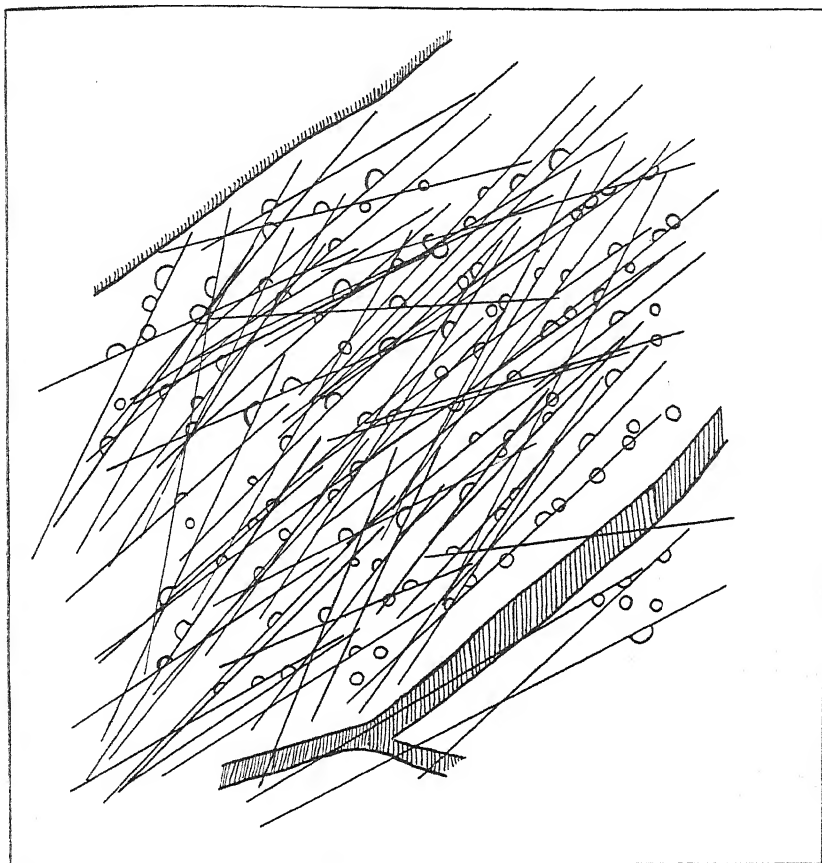


FIG. 11. *F. verna*. Detail of area no. 5, Fig. 10. Stomatal initials shown as circles, mature stomata as semicircles.

larger radial veins, though considerable, was not so high as in the immature leaf.

As the vein reticulum is more highly developed in the older leaf, some attempt was made to determine the 'resultant' vein direction for each of the areas studied. The vascular system was drawn in outline, and the width of each vein was measured (in arbitrary units). A series of 'parallelograms of forces' was constructed—the length (l) of a given vein being multiplied by its width (d) so as to make some empirical allowance for its effective size. The lines representing $l \times d$ were drawn at angles equal to those which the veins made with each other. Thus, by obtaining a single resultant for two veins, and then a second resultant between this

and another vein, by repetition of this process, eventually a single resultant was obtained, by which was taken into account the length, direction, and width of all the veins of the area. The angles made by all the stomatal

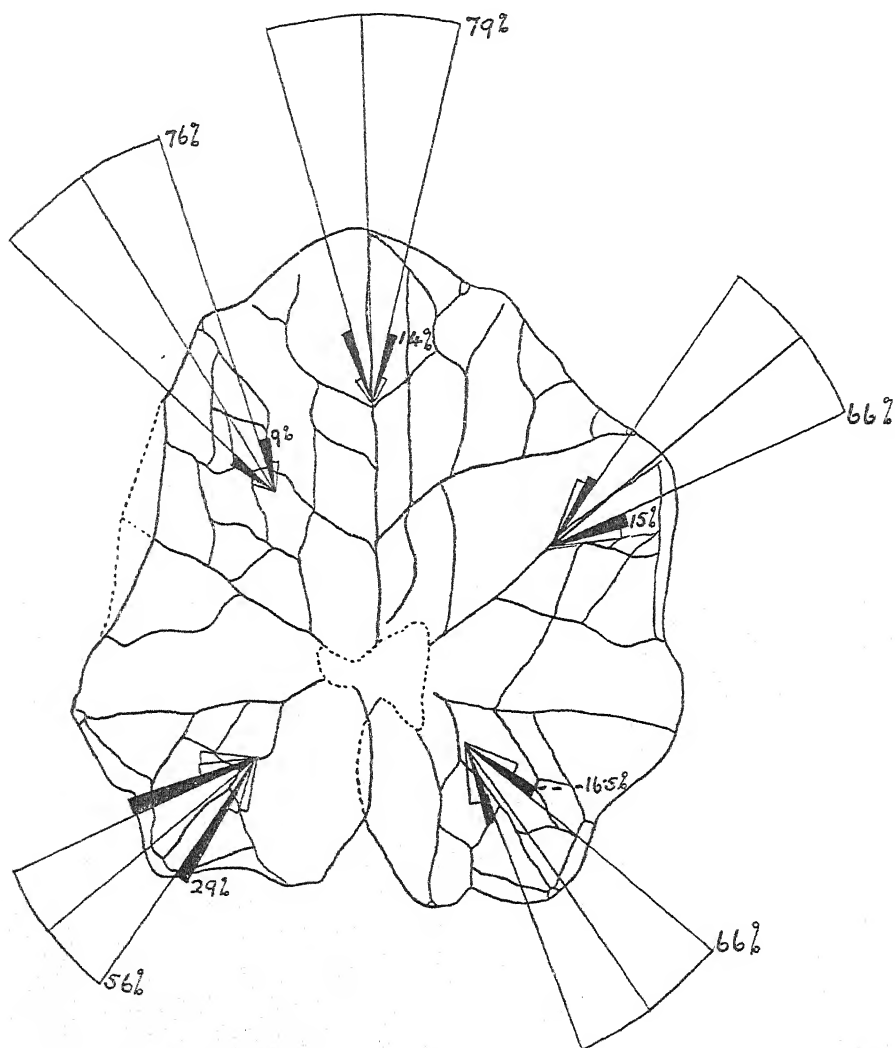


FIG. 12. *F. verna*. Young leaf. The length of each 'cone' is proportional to the number of stomata, the orientation of which falls within the angle of the cone. In each case the median line is the reference line with which stomatal orientation was measured.

axes were redetermined, using the resultants as 'base lines', and with these a correlation was obtained as high as with the radial veins in the young leaf.

The angles made by the stomatal axes with the radial veins are given in Table V A, and those made with the resultants in Table V B. Figs. 13 A

TABLE IV.

Immature leaf of Ficaria verna.

Sample No.	Total no. of stomata.	Divergence from nearest radial vein.							
		0-5°	5-10°	10-15°	15-20°	20-25°	25-30°	30-35°	35°<
1	36 + (27 initials)	8	6	6	10	2	1	2	1
	percent.	22.3	16.7	16.7	27.7	5.5	2.8	5.5	2.8
2	98 + (67 initials)	29	36	11	9	3	3	5	2
	percent.	29.5	37.0	11.2	9.2	3	3	5.1	2
3	44 + (18 initials)	20	6	9	6	1	1	—	1
	percent.	45.5	13.6	20.4	13.6	2.2	2.2	—	2.2
4	65 + (24 initials)	18	15	10	10	6	3	1	2
	percent.	27.7	23.0	15.4	15.4	9.2	4.6	1.5	3.0
5	85 + (21 initials)	21	18	17	14	5	7	2	1
	percent.	24.7	21.2	20.0	16.5	5.9	8.25	2.35	1.2
Totals		96	81	53	49	17	15	10	7
Percentages		29.3	24.6	16.1	14.9	5.2	4.6	3.1	2.1
		70 per cent.				9.8 per cent.			

TABLE V A.

Correlation with Radial Veins.

	0-5°	5-10°	10-15°	15-20°	20-25°	25-30°	30-35°	35-40°	40-45°	45-50°	Tot.
Position I	20	9	7	7	0	3	4	1	—	—	51
Percent.	39.2	17.6	13.7	13.7	0	5.8	7.7	1.96	—	—	
70.5 per cent.											
Position II	1	3	0	9	4	9	4	3	6	2	42
Percent.	2.38	7.15	0	21.4	9.6	21.4	9.6	7.15	14.3	4.75	
9.53 per cent.											
Position III	8	7	10	5	—	—	—	—	—	—	30
Percent.	26.6	23.3	33.3	16.6	—	—	—	—	—	—	
83.2 per cent.											
Position IV	8	13	9	6	5	3	0	2	1	—	47
Percent.	17	27.7	19.5	12.8	10.6	6.4	0	4.26	2.1	—	
64.2 per cent.											
Position V	1	5	9	5	2	2	4	1	2	1 at 75	32
Percent.	3.1	15.6	29.05	15.6	6.25	6.25	12.5	3.1	6.25	3.1	
47.75 per cent.											

and 13 B respectively show a sample patch (No. 5), and the determination of the resultant for that area. It must be emphasized that this 'resultant' method conveys in a clumsy way what is evident on an actual examination of the leaf—i.e. that a high correlation exists between stomatal orientation and the *general direction* of the veins of a given area.

The resultants differed from the direction of the radial veins by the following amounts:

Position I negligible	Position II 14°	Position III 3°	Position IV 8°	Position V 13°
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TABLE V B.

Correlation with 'Resultant' of Veins.

	0-5°	5-10°	10-15°	15-20°	20-25°	25-30°	30-35°	35-40°	40-45°	45-50°	Tot.
Position I	20	9	7	7	—	3	4	1	—	—	51
Percent.	39.2	17.6	13.7	13.7	—	5.8	7.75	1.96	—	—	
	70.5 per cent.										
Position II	9	6	10	4	3	5	4	1	—	—	42
Percent.	21.4	14.3	23.8	9.6	7.15	11.9	9.6	2.38	—	—	
	59 per cent.										
Position III	9	9	8	1	3	—	—	—	—	—	30
Percent.	30	30	26.7	3.3	10	—	—	—	—	—	
	86.5 per cent.										
Position IV	16	12	6	6	3	1	2	1	—	—	47
Percent.	34	25.6	12.8	12.8	6.4	2.1	4.2	2.1	—	—	
	71 per cent.										
Position V	14	6	3	2	4	1	1	—	—	1	32
Percent.	43.7	18.7	9.37	6.25	12.5	3.1	3.1	—	—	3.1	
	72 per cent.										

It is very difficult to obtain experimentally any direct evidence of tensions in the young leaf, and those present in the adult leaf are of little value as indications of the conditions obtaining in the earlier stages.

Experiments were made in growing various young leaves under tension, but they usually were too delicate to support the attachment of any very considerable weight, and no definite results were obtained.

Attempts to obtain evidence of the direction of expansion from cracks developing when the young leaf or stem was painted with Indian ink were likewise unsuccessful. Sliding growth occurred between the dried ink and the leaf surface. The method does show, however, differences between the *amount* of expansion of the epidermis over veins and in vein-free areas, though it gives no indication of the extent or direction of the differences.

It was thought that the examination of periclinal chimaeras might throw more light on the question as to whether the veins of a given leaf really determine stomatal orientation, or whether the epidermis itself

inherently contains the controlling factors. If the vein development is the main cause of the determining factor, or factors, the stomatal orientation of the chimaeras should follow closely that of the vein parent. During the

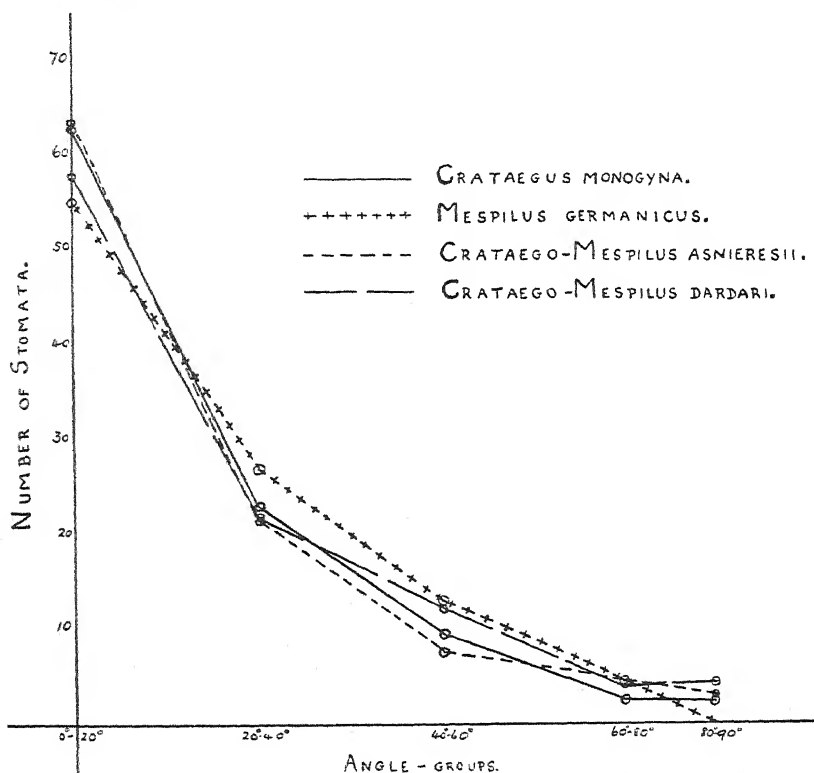


FIG. 14. Explanation in text.

investigation of literature referring to graft hybrids and chimaeras it was found that Linsbauer (12) mentions the importance of periclinal chimaeras as providing evidence of the influence of inner tissues on the outer (see also p. 473).

Leaves of *Crataegus monogyna* and *Mespilus germanicus*, and of the two chimaeras from them, *Crataego-mespilus asniieresii* and *Crataego-mespilus dardari* were cleared in Eau de Javelle and investigated similarly to those of *Ficaria*. The stomatal orientation was measured in degrees in relation to the nearest veins, and then the stomata were classified into 'angle-groups', so that the results might be expressed graphically (Fig. 14).

It is seen that the two hybrids resemble the vein parent (*Crataegus*), and that the most divergent is the skin parent (*Mespilus*). The hybrid which most nearly approaches *Mespilus* is *C.-m. dardari*, with two layers of *Mespilus* tissue over the *Crataegus* core.

Material of *Solanum* graft hybrids was obtained through the courtesy of the John Innes Horticultural Research Institution. *Solanum lycopersicum* and *S. luteum* were the parents of the two chimaeras chosen, one of which

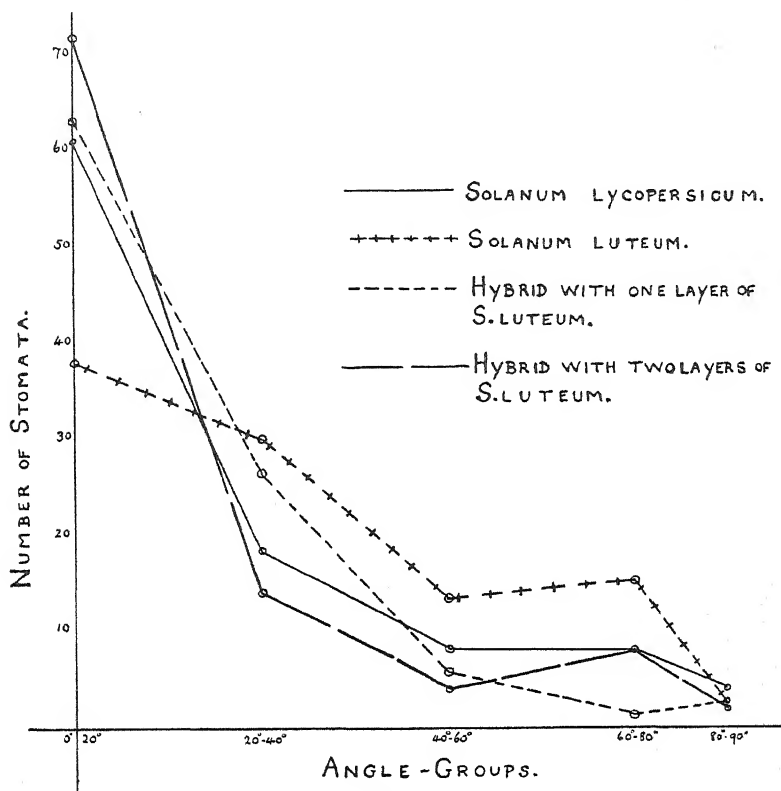


FIG. 15. Explanation in text.

had one layer of *S. luteum* and the other two layers of *S. luteum* over a core of *S. lycopersicum*.

The methods adopted were the same as in the previous example, and the results are shown graphically in Fig. 15.

The stomatal frequency of the hybrid with one layer of *S. luteum* is very low, and a correspondingly greater number of samples was taken.

The two hybrids and *S. lycopersicum* have very similar proportions of parallel orientation, and the most divergent of the four is the pure 'skin parent', *S. luteum*.

Thus the evidence from periclinal chimaeras, in so far as it may be relied upon, supports the idea that stomatal orientation is intimately associated with the direction of the underlying vascular tissue, rather than being an intrinsic character of the epidermis itself.

A number of young buds have been embedded and the epidermides examined in surface section.

Suaeda fruticosa is difficult to examine satisfactorily, as the leaves bend over sharply at the apex, but it has been possible to establish that regular rows of epidermal cells exist from the youngest stages, and that when the stomata are formed they are cut off in intercalary positions in the rows; in this species stomatal orientation is regular and transverse to the long axis of the leaf. Cell division appears to proceed quite evenly throughout the rows of young epidermal cells, thus no evidence was obtained for the occurrence of 'mother-cells', which by successive transverse divisions might have given rise to the linear arrangement in the mature leaf.

Prunus lauro-cerasus shows very few stomata until the leaf is quite large (5–10 mm.). The stomata in the youngest leaves are nearly all parallel with the veins which are then present. The epidermal cells are formed in groups from epidermal 'mother-cells', but these 'mother-cells' show no regular arrangement.

In *Sambucus nigra*, stomata with a well-developed pore occur side by side with circular initials with no cross wall; here also the stomata are mostly parallel with the veins, but there is no evidence for the occurrence of 'epidermal mother-cells'.

Cell division seems to proceed evenly throughout the epidermal layer. In sections of very young leaves it is possible to make out an ill-defined linear arrangement, but in slightly older leaves this is entirely lost except in regions over and near to the veins.

Surface sections of *Ficaria verna* show that the meristematic epidermal cells tend to be arranged in rows over the veins and irregularly over the remainder of the leaf area.

DISCUSSION.

From the data here recorded it is evident that in many plants there is a correlation between the orientation of the stomata and the course of the underlying vascular system. It seems likely that the mechanical influence of the veins on the epidermis is of importance in producing the correlation, and in this connexion it is of interest to recall the work of Kny (9), on the factors which determine the orientation of the cross walls in dividing plant cells.

Kny's conclusion is 'that growth in as far as no other forces act in a contrary direction, will be increased in the direction of tension, and perpendicular to pressure—the cell divisions "endeavour" to place their separating walls in the direction of pressure and at right angles to the direction of tension'.

He further found that the direction of the incident light largely determined the direction of the first cross walls in germinating *Equisetum* spores,

but this factor can rarely be operative in developing leaves, as stomata are usually determined as to orientation, while the leaf is still rolled or protected by other structures.

Avery (2) states that tensions exist in young tobacco leaves, and that it appears to be the inner tissues which give the impetus for expansion. He finds that the cell divisions cease first in the epidermis, but that as these cells continue to expand after divisions cease in the mesophyll, the cells of the latter are dragged apart and the formation of air spaces results.

His observations agree with those of the present writer as to the early differentiation of stomata at the apex of the leaf, and it would appear from his description that their differentiation in other parts is concurrent with the development of air spaces in the mesophyll. The last meristematic activity is seen in the development of connecting veins in the middle mesophyll.

In a recent paper on the embryonic leaf Smith (16) outlines the development of the leaves of a number of common American trees and shrubs. It is emphasized that the tissue of the embryonic leaf is nearly homogenous in the bud, and that not until the time of expansion does much differentiation occur. It is about this time that stomata are commonly formed.

Lee and Priestley (11) consider that when the cuticle is a relatively rigid covering, it largely determines the small size and regular shape of the epidermal cells, which commonly accompany a thick cuticle.

Linsbauer (12) states that the size and form of epidermal cells are partly determined by heredity, partly by the influence of neighbouring cells, and partly by external conditions. He omits a consideration of the effect of the cuticle. He considers that the differences in shapes of epidermal cells over veins, palisade, and spongy tissues point to the influence of internal factors on epidermal structure. This author further indicates that the study of periclinal chimaeras may give an indication of the existence of correlations between the mesophyll and the epidermis, and proceeds to a consideration of *Cytisus-Laburnum*, *Solanum* graft-hybrids, and the *Crataego-mespilus* combinations.

From these plants evidence is adduced that the inner tissues are effective in modifying the structure of the outer, and it is concluded that similar factors must be operative in ordinary plants where the effects are not so readily demonstrable.

Various theories to explain the development of waving in the lateral walls of epidermal cells have been put forward by Vesque (20, 21), Zimmerman (25), Berthold (5), Ambronn (1), and Brenner (7), but none of their views seem to suggest that the orientation of the stomata would be altered during the development of waving. Cp. Salisbury (15, p. 539).

It may be concluded that the conditions obtaining in the developing

expanding leaf, at the time of the formation of stomatal initials, are such as make it probable that tensions are set up in the epidermis by the adjacent tissues, and one might expect the veins to play a large part in this development of tension, since they are the least plastic part of the leaf at all stages of its development.

TABLE VI A.

Stomata Parallel to One Another and to the Long Axis of the Leaf, Leaf-lobe, or Stem.

- | | |
|---------------------------------|--|
| 1. Ranunculaceae. | <i>Aconitum napellus</i> , <i>Nigella</i> , <i>Ranunculus gramineus</i> , <i>R. flammula</i> , <i>R. lingua</i> , <i>R. sceleratus</i> , <i>R. aquatilis</i> (land form), <i>Paeonia</i> sp. |
| 2. Leguminosae. | <i>Vipinaria</i> , <i>Jacksonia</i> (at bottom of long pit), <i>Cytisus</i> , axis of <i>Phaseolus</i> &c., Stipules of <i>Vicia faba</i> .
In Mimosae 'on narrow pinnules and phyllodes the stomata are frequently parallel to one another'. |
| 3. Melastomaceae. | <i>Microlicia</i> sp., <i>Pterolepis</i> , <i>Osbeckia</i> and <i>Dissothis</i> . |
| 4. Bruniaceae. | Stomata in 1 row, <i>Brunia lanuginosa</i> ; in 2 rows, <i>B. arachnoidia</i> , <i>Titmannia laterifolia</i> , <i>Audouinia capitata</i> ; in 4-5 rows, <i>Linconia cuspidata</i> , <i>Berardia paleacea</i> , <i>Lonchostoma</i> . |
| 5. Cactaceae. | <i>Lepismium</i> , <i>Epiphyllum</i> , <i>Opuntia</i> . |
| 6. Umbelliferae. | <i>Eryngium</i> (sp. with monocot-like leaves). Leaf sheaths of various sp. |
| 7. Halagoraceae. ¹ | <i>Callitriche stagnalis</i> , floating and immersed leaves. |
| 8. Rubiaceae. | <i>Galium verum</i> , <i>G. cruciata</i> , a high proportion parallel to midrib of all 'leaves' at a node. |
| 9. Epacridaceae. | 'Epidermal cells elongated and stomata generally parallel to midrib'. |
| 10. Plumbaginaceae. | 'Species of <i>Statice</i> and <i>Acanthalimon</i> with narrow leaves.' |
| 11. Loganiaceae. | <i>Polypleurum procumbens</i> 'probably connected with the acicular shape of the leaf'. |
| 12. Illecebraceae. ¹ | <i>Scleranthus</i> , <i>Habrosia spinulifera</i> , <i>Anychia dichotoma</i> , <i>Gymnocarpus fruticosum</i> . |
| 13. Chenopodiaceae. | <i>Polycnemum</i> . |
| 14. Nepenthaceae. | 'Stomata on lamina approximately parallel to midrib.' |
| 15. Proteaceae. | 'Most species' (except where they occur in pits). |
| 16. Santalaceae. | <i>Arjona</i> , <i>Myzodendron</i> . |
| 17. Lythraceae. | <i>Diplosodon</i> (sp.) <i>Lythrum</i> , <i>Heimia</i> , <i>Nesaea</i> , <i>Pleurophora</i> . |
| 18. Saxifragaceae. | In patches of <i>Chrysosplenium oppositifolium</i> and on linear leaves of other sp. |
| 19. Rosaceae. | Stipules of <i>Prunus lauro-cerasus</i> . |
| 20. Compositae. | Leaf-segments of <i>Achillea millefolium</i> . |

Most Monocotyledons, Cycads, Cordaitaceae and Coniferae.

If, in fact, the epidermis is tending to expand more rapidly than the underlying bundle tissues, it will tend to be thrown into folds as if under a pressure operating in the direction of the veins. One would, therefore, expect the division walls of stomatal initials to be formed parallel with the veins, if Kny's results can be applied here. When the relative rates of expansion instanced above are reversed, one might anticipate a transverse orientation of the stomata—but while this may be the cause of transverse

¹ So given by Solereder (17).

orientation in some species, what has been observed suggests that often the regularity of the divisions of the meristematic epidermis may explain this adult condition.

In the light of the hypothesis stated above one can understand that the proportion of 'regular' stomata on the young leaflets of *Sambucus*

TABLE VI B.

Stomata Parallel to One Another and at Right Angles to Long Axis of Leaf, Leaf-lobe, or Stem.

1. Tamaricaceae.	<i>Tamarix, Hololachne, Racumuria Myricaria.</i>
2. Leguminosae.	<i>Krameria triandra, Carmichaelia</i> (stem and leaves), <i>Alhagi, Eutaxia, Latrobia tenella</i> (leaves), <i>Daviesia</i> (axes), <i>Anarthrophyllum</i> (leaves).
3. Bruniaceae.	<i>Staavia radiata, S. capitella, Brunia palustris.</i>
4. Empetraceae.	<i>Empetrum</i> (leaves).
5. Cactaceae.	<i>Pfeffera</i> and <i>Rhipsalis.</i>
6. Ficoideae. ¹	<i>Mesembryanthemum adscendens, M. uncinatum.</i>
7. Epacridaceae.	<i>Lysinema.</i>
8. Chenopodiaceae.	'Very common on leaf and axis.' <i>Camphorosma, Monospeliacum, Echinopsilon, Halogeton, Salsola kali, S. soda, Suaeda fruticosa, S. maritima, Traganum nudatum.</i>
9. Batidae.	<i>Batis maritima.</i>
10. Lauraceae.	<i>Cassytha.</i>
11. Loranthaceae.	<i>Arceuthobium, Antidaphne, Lepidoceras, Loranthus, Nuytsia</i> (leaves and axis), <i>Viscum.</i>
12. Santalaceae.	<i>Exocarpos, Leptomeria, Omphacomeria, Orysis, Chloretum, Fusanus, Henslowia, Myoschilos, Santalum, Thesium</i> (in longitudinal rows), <i>Antkrobolus.</i>
13. Euphorbiaceae.	<i>Euphorbia alluandi, E. leucadendium, E. onocladia.</i>
14. Casuarinaceae.	<i>Casuarina.</i>
15. Liliaceae.	<i>Phyllesia, Lapageria</i> (and hybrid <i>Phyllageria).</i>
16. Podocarpaceae.	<i>Podocarpus, sp.</i>

nigra may be high, while in the mature leaf it is much lower. If the above premises are correct, the veins will only be directionally operative so long as the epidermis and internal tissues have not attained tensional equilibrium, and will only affect the orientation of stomata developing during the same period. Thus one can readily appreciate that stomata which stand in no regular relationship to a neighbouring vein may well have been developed after its period of major expansion, or in relation to another vein which subsequently may not be the nearest to them.

Where the whole of the vein system tends to be parallel or in the same general sense, and where reticulation is only slight, the high proportion of parallel stomata remains evident in the adult lamina, since under these conditions later developed stomata are laid down and orientated in the same way as were the earlier ones. This is the condition which obtains in most of the species mentioned in Table VI A, the leaves of which are often acicular.

¹ So given by Solereder (17).

It may be mentioned that the results obtained in some detail on young leaflets of *Sambucus* have been confirmed by general observation on a series of leaves of different ages from a young shoot of *Ligustrum vulgare*, for a shrubby species of *Lonicera*, and for *Prunus laurocerasus*.

SUMMARY.

1. Evidence is furnished for the existence in many leaves of a marked correlation between the direction of the long axes of the stomata and that of the underlying vascular tissue.

2. Data are presented on the orientation of the stomata of young and old leaves of *Sambucus nigra* and of *Ficaria verna*. Orientation parallel to the veins is shown to be characteristic of the earlier differentiated stomata, whilst those differentiated later often have a more irregular orientation.

3. Two pairs of periclinal chimaeras examined showed more resemblance to the 'vein-parent' than to the 'skin-parent' as regards stomatal orientation.

4. It is concluded from several lines of evidence that there is often a correlation between stomatal orientation and vein-direction, which may exist in net-veined leaves, where the relationship is not immediately apparent.

5. It is suggested that the stresses set up in the epidermis consequent upon the differences in rate of development of the bundle tissues and mesophyll on the one hand, and of the epidermis on the other, may be an important factor in determining stomatal orientation.

The writer wishes to express his keen appreciation of the continued advice and encouragement, throughout the course of this work, given to him by Professor E. J. Salisbury, F.R.S.

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The Problem of Immunity to Wart Disease [*Synchytrium endobioticum*, (Schilb.) Perc.] in the Potato.

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DURING the period 1915-27 potato seedlings were raised at the John Innes Horticultural Institution in carrying out a research on the problem of the inheritance of resistance to late blight (*Phytophthora infestans*) in the potato. Incidentally these seedlings were available for the wart disease trials which were being carried on at Ormskirk, and from 1918 onward seedlings were sent to be tested.

Many individuals of the various progenies during the first year of their growth at Merton, Surrey, became infected with diseases grouped under the terms 'curl', 'mosaic', 'leaf-roll', &c., and during their subsequent trial at Ormskirk were often classed as 'too poor' to give a definite result in these trials, a certain vigour of growth being held a precedent to infection by wart disease. Positive evidence of susceptibility was accepted during the first year of trial, but if no infection was seen, the seedling tubers were harvested and tested again the following year; only by escaping infection for two seasons was a seedling pronounced to be immune.

Except for a series of articles in the 'Gardeners' Chronicle' (1), 1921, the results of the annual tests of these seedling progenies were not published, although they were briefly embodied in the yearly report presented to the Council of the John Innes Institution in the period 1918-27.

In the articles published in the 'Gardeners' Chronicle' it was suggested, tentatively, that susceptibility would prove to be dominant. A further year's work showed that this view could not be sustained, and that a much more complicated story appeared to be involved. At this time also Salaman and Lesley (4, 5) were engaged with the problem of wart disease resistance in potatoes, and published papers from which it could be seen that a solution of the problem of the inheritance of susceptibility, or resistance, envisaged the assumption of a considerable number of factors, and that these varied according to the varieties of potatoes used as progenitors. As all workers have found, sterility, particularly a lack of pollen, was a

formidable difficulty in potato-breeding, and prevented a straight progeny being raised in many instances, where if such had been possible, a definite conclusion could easily have been reached. Further, progenies arising from selfings were always found to be unprofitable, inasmuch as the plants had no constitution or vigour of growth and generally failed, forming that class of 'too poor' individuals about which the evidence for infection was inconclusive.

A considerable body of results, however, was obtained during the period of the tests and since the recent appearance of a paper by Lunden and Jørstad (3) it seemed desirable to publish such results more fully, so that the whole evidence could be reviewed; at any rate the additional facts may strengthen the chain of evidence for or against views on which agreement has not been reached.

In all, six classes of results were obtained as a result of selfings and crossings, directly, indirectly, and where possible reciprocally, but the material cannot be handled as one would like, and conclusions must be based on evidence which is cumulative, rather than on the evidence obtained by direct and exact breeding of individual varieties. I have taken the liberty of remodelling the Tables taken from the paper by Lunden and Jørstad, so as to show at a glance the results obtained in each of these six classes, and present them in summarized form, easy of comparison with those which I obtained. Salaman and Lesley's results are interpolated with comments as occasion arises. This is made necessary if the three groups of researches are to be presented as a comparative record.

CLASS I. IMMUNE, SELFED.

Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
Leinster Wonder	18	4	14	—	Rep. Int. Potato Conf., 1921. Salaman and Lesley.
" "	10	5	5	—	'Journ. Gen.', 1923. Salaman and Lesley.
" "	8	3	5	—	" " " "

In 1921 Salaman and Lesley interpreted the result on a 1:3 basis, but the final results shown in 1923 and aggregated led them to conclude that the variety Leinster Wonder segregated on a 7:9 basis, susceptible to resistant seedlings.

Favourite. (Natural berry)	17	2	14	1	'Gardeners' Chronicle', 1922. Cuthbertson.
Edzell Blue. (Natural berry)	28	6	22	—	Rep. Int. Pot. Conf., 1921, Salaman and Lesley.
Edzell Blue	13	1	12	—	'Journ. Gen.', 1923. Salaman and Lesley.
" "	15	5	10	—	" " " "

The two latter results were put together in 1921, and the progenies have apparently been derived from naturally set fruit. The segregation was regarded as based on a 1:3 ratio. Although many attempts to self the varieties Edzell Blue and Favourite were made, I was not successful on any occasion in obtaining seed. In the South, at Merton, Surrey, it was frequently noticed that, owing no doubt to the rapid growth of the haulm, the quantity of flowers produced on the different varieties was less than when the varieties were grown further north; also the quantity of pollen borne by the flowers of some varieties was usually small or none at all, when further north the same varieties evidently produced pollen in varying degree.

Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
Majestic	25	9	10	6	'Journ. Gen.', 1923. Salaman and Lesley.
Majestic (Natural berry)	63	22	38	3	'Gardeners' Chronicle', 1922. Cuthbertson.
12/22. Majestic	25	5	19	1	Ormskirk. Collins.

Salaman and Lesley took the first two results as almost identical, combined the numbers to 31 S:48 R, and approximated the figures to a 7:9 ratio, suggesting the existence of two factors which produced immunity only when both were present.

The result which I obtained with Majestic, however, more nearly approximated to the 1:3 ratio, and the crossing results between Majestic and other immune varieties lend additional weight to this assumption. See later Tables.

5/22. Flourball	25	4	20	1	Ormskirk. Collins.
8/24. "	9	1	8	—	" " " " " "
"	43	13	30	—	'Journ. Gen.', 1934. Lunden and Jørstad.

Lunden and Jørstad regard this result as a segregation of 1:3. Salaman and Lesley have no direct result from the variety Flourball. A closer approximation to the 1:3 ratio is reached if the three results are aggregated, although it must be observed that my figures are divergent in degree.

Other direct results, ten in number, from immune varieties selfed are given by Lunden and Jørstad. The progenies of these are approximated to the 1:3 ratio, S:R, but it should be mentioned that all except Flourball and Tannenburg (not identified) were derived either directly or indirectly from the variety Jubel. In all, immunity is assumed to be dependent on a single factor, in heterozygous condition and independent of any other factor producing immunity.

Other results from immune varieties have been :

Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
Priory Queen ¹	8	0	8	—	'Gard. Chron.', 1922. Cuthbertson.
Favourite ¹	17	2	14	1	" " "
Admiral ¹	62	5	48	9	" " "
Climax	29	2	26	1	" " "
Templar	38	5	28	5	" " "

CLASS II. IMMUNE × IMMUNE.

The earliest recorded results are those of Salaman and Lesley, and may be briefly brought together here, with other results obtained since.

Kerr's Pink × Champion 2	79	3	76	—	Rep. Int. Pot. Conf., 1921, and 'Journ. Gen.', 1923. Salaman and Lesley.
Golden Wonder × Leinster Wonder	21	7	14	—	Rep. Int. Pot. Conf., 1921. Sala- man and Lesley.
Golden Wonder × Leinster Wonder ²	27	9	18	—	'Journ. Gen.', 1923. Salaman and Lesley.

In the result Kerr's Pink × Champion 2, the ratio of susceptible to immune seedlings is approximated to 1:15, involving two factors independently capable of inducing immunity.

My results in this class are tabulated below, and were obtained at the Ormskirk trials over a period of years. The results were sent to me at the close of each season by the Superintendent of the trials, Mr. H. Bryan. Mr. Bryan was quite unaware of the origin of the progenies which were submitted to him under numbers.

8/17. Defiance × Lein- ster Wonder	5	—	5	—	A rarity among experimental results.
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Lunden and Jørstad record a similar result from Irish Cobbler × Jubel with a progeny of nineteen seedlings.

6/20. Edzell Blue × Majestic	27	9	18	—	Ratio approximations of $\begin{cases} 1:3. \\ 3:5. \end{cases}$
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Lunden and Jørstad with Edzell Blue × Jubel obtained alternative ratios of 1:7 and 3:13. See their Tables. This crossing, these authors state, should segregate 1S:3R as both varieties, Edzell Blue according to

¹ These varieties are probably synonyms and identical with Abundance according to Salaman and Lesley, who aggregating the figures (7S:70R) have regarded the result as suggestive of a 1:15 ratio, but since these progenies as well as those of the other varieties listed were derived from seed of naturally set berries, the results must be regarded with caution. Abundance × Jubel by Lunden and Jørstad gave a 1:3 ratio, and in my results, Favourite × Majestic and Abundance × Flourball progenies approximate to a 1:3 ratio, susceptible to immune. I have already mentioned that I was unable to self the variety Favourite, syn. Abundance.

² These figures must be the final result for this cross; in the 1921 paper it was suggested that the ratio involved was 1:3 and I presume, since no further observation was made on the final result, that this ratio stands.

Salaman and Lesley, are heterozygous for one immunity factor, but the result showed a deviation from the expected ratio nearly 4.5 times the probable error. The result from Edzell Blue given by Salaman and Lesley might be questioned, since the progeny tested was obtained from naturally set seed, but there is some evidence confirmatory of the heterozygosity in one factor of Edzell Blue, in my results from Edzell Blue \times Majestic and Edzell Blue \times Edgecote Purple.

	Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
3/21.	Snowdrop \times Majestic	16	3	7	6	Four tests in differing years. In the aggregate 19S:56R, a close approximation to a 1:3 ratio but family 5/24 is divergent.
16/22.	" "	27	8	17	2	
5/24.	" "	14	1	13	—	
11/25.	" "	26	7	19	—	

Majestic selfed gave an approximate ratio of 1S:3R.

5/21.	Golden Wonder \times Majestic	12	2	9	1	Calculations based on aggregate figures of 9:44 are, Ratio 1:3 or 13.25:39.75, " 1:7 or 6.6:46.2.
11/22.	" "	20	2	12	6	
13/24.	" "	30	5	23	2	
9/21.	Favourite \times Majestic	8	2	6	—	9S:27R is needed for a 1:3 ratio as against a tested aggregate of 7S:29R; the third result is divergent.
9/22.	" "	16	4	11	1	
12/24.	" "	14	1	12	1	
1/22.	Bishop \times Flourball	21	3	14	4	Approximates to 1S:3R.
7/24.	" "	13	4	9	—	Flourball selfed approximates 1:3.
4/22.	Abundance \times Flourball	44	10	30	4	Ratio of 1S:3R.

With Lunden and Jørstad, Abundance \times Jubel gave 1S:3R.

6/22.	King George \times Flourball	22	4	14	4	Approximates 1S:3R.
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Lunden and Jørstad using King George \times Centifolia obtained 1S:1R.

6/24.	Bishop \times Majestic	15	5	10	—	Approximates 1S:3R.
2/22.	Immune Ashleaf \times Flourball	16	1	10	5	On two separate occasions a single susceptible seedling was recorded giving a total of, 2S:30R, ratio 1:15.
11/24.	" "	21	1	20	—	

The last result accords with one found by Lunden and Jørstad, viz. Kerr's Pink \times Jubel and one by Salaman and Lesley, namely, Kerr's Pink \times Champion 2. The number of seedlings is small in my results, but quite naturally, I think, one wonders if some mistake may have been made in this one suspect in each family. It is rather remarkable that a pure immune should be of such rarity, although the close inbreeding in the production of modern varieties of potatoes must be recognized. However, excluding family 8/17 where no mixed progeny resulted, but including the

weighted results from families 2/22, 11/24, and Salaman and Lesley's single result, Golden Wonder \times Leinster Wonder, the totals are 353 seedlings, 81S:272R, and no ratio fits closer than one of 1:3. The legitimacy or wisdom of including widely divergent individual results may be questioned, but the cumulative evidence for a simple ratio is very strong, alternative ratios of 3:5, 1:7, 1:15, 3:13 indicated in Lunden and Jørstad's individual results being widely divergent. Excluding the weighted results in families 2/22, 11/24, the totals are 321 seedlings, 79S:242R a much closer approximation to a 1:3 ratio (80S:241R). If we turn to the group of results under Snowdrop \times Majestic or Favourite \times Majestic it could be concluded from the one divergent set of figures in each that a 1:15 ratio was involved, but the evidence obtained in other years from the same crossing, puts this conclusion out of court, for the sum totals in the groups indicate the simpler ratio.

CLASS III. SUSCEPTIBLE, SELFED.

As with immune varieties, very few progenies resulting directly from selfing have been obtained and tested out. Salaman and Lesley used Edgecote Purple, and Lunden and Jørstad used Centifolia.

Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
Edgecote Purple	42	22	20	—	Rep. Int. Pot. Conf., 1921. Salaman and Lesley.
" "	31	27	2	2	'Journ. Gen.', 1923. Salaman and Lesley.

Salaman and Lesley were not satisfied with the first testing, for they considered that the resistant seedlings were probably excessive owing to 'poor plants', and in the later result that the two seedlings returned as resistant were too feeble to contract disease. The earlier factorial explanation of Edgecote Purple given by these authors may be ruled out and the final result taken, namely, that Edgecote Purple on selfing gives seedlings all of which are susceptible. This view is strengthened by the additional evidence of the behaviour of Edgecote Purple in crossings with other susceptible varieties and dealt with later.

Myatt's Ashleaf	22	9	11	2	'Journ. Gen.', 1923. Salaman and Lesley.
" "	28	17	11	—	" "

It was assumed on the basis of these figures 26S:22R that a 9:7 ratio was involved, and from the differing results with Edgecote Purple and Myatt's Ashleaf concluded that there were two groups of susceptible varieties.

(a) those that produce only susceptible seedlings on selfing,

(b) those that produce both susceptible and resistant seedlings on selfing.

Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
President (Natural berry)	29	15	14	—	'Gard. Chron.', 1929. Cuthbertson.

The variety President is variously known as Paul Kruger, Iron Duke, Scottish Farmer, and is most liable to virus troubles. The wart test is open to the objection that the pollination was not controlled. On the other hand, President was likened to Myatt's Ashleaf by Salaman and Lesley, inasmuch as it gave both susceptible and resistant seedlings.

For my results I could use only the offspring from two proved susceptible seedlings, selfed.

6/25. Seedling, 5/21, 57.	32	32	0	—	Ormskirk. Collins.
8/25. „ 9/22, 49.	29	26	3	—	„ „

Both seedlings were derived from crossing two immune varieties, the first from Golden Wonder \times Majestic and the second Favourite \times Majestic. The figures are taken to indicate complete susceptibility of the offspring, and the purity of the recessive susceptible which falls out upon the crossing of two immunes heterozygous in one factor for immunity. It may be said that these two seedling tests were designed and made as a check upon the Ormskirk returns.

In the only test made by Lunden and Jørstad, Centifolia selfed, gave a progeny which consisted wholly of susceptible seedlings.

CLASS IV. SUSCEPTIBLE \times SUSCEPTIBLE.

Again in this group of tests few results are available from well-known varieties. There is a small set from the investigations of Salaman and Lesley, in which the two types of susceptibles assumed by these authors are involved, and which to all intents confirm the assumption, by the differing results.

May Queen \times Edgecote Purple	33	31	1	1	'Journ. Gen.', 1923. Salaman and Lesley
Eclipse \times Sharpe's Victor	6	6	0	—	„ „ „ „
Myatt's Ashleaf \times Edgecote Purple	22	20	2	—	„ „ „ „
„ „	37	28	9	—	„ „ „ „
Edgecote Purple \times Myatt's Ashleaf	39	31	5	3	„ „ „ „
„ „	53	43	8	2	„ „ „ „

Salaman and Lesley suggested that Myatt's Ashleaf was in reality an immune variety, immunity being inhibited by one or more factors. The

result of the selfing was regarded as a 9:7 ratio; the matings with Edgescote Purple, which seems beyond doubt a pure susceptible type, widely diverge from the 3:1 ratio assumed. The first two results appear to afford a clear indication of other pure susceptible varieties.

As far as like matings were made by Lunden and Jørstad, the progenies of any two susceptible varieties were wholly susceptible. This proved to be so in four tests, using *Centifolia* as a male parent with four different varieties as female parents.

My own results in this class are as follows:

Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
2/25. British Queen × Edgescote Purple	24	23	1	—	Ormskirk. Collins.
3/25. President × Edgescote Purple	27	27	0	—	„ „
3/18. British Queen × 30/16, 47	4	4	0	—	Seedling 30/16, 47 was proved S. at Ormskirk.

Under the circumstances of trial I think these three results show that the three varieties, British Queen, President, and Edgescote Purple, are pure susceptible varieties. This would confirm the evidence for Edgescote Purple, uphold my objection to President as giving equality, immune and susceptible in its progeny as suggested by the progenies derived from the seed of naturally set fruit, and add the well-known variety British Queen to the list of susceptible varieties giving wholly susceptible stock, if it could be self-fertilized.

The suggestion made by Salaman and Lesley, that President is a susceptible variety similar in kind to Myatt's Ashleaf cannot be maintained.

CLASS V. IMMUNE × SUSCEPTIBLE.

In this class it will be more convenient to tabulate my results first, and make the necessary comments. The following were recorded from Ormskirk.

2/17. Defiance × Edgescote Purple	4	3	1	—	An early result which contributed to the earlier conclusion as to dominance.
12/18. Templar × 30/16, 47	3	—	3	—	Templar was raised by Dr. Wilson of St. Andrews.
7/20. Edzell Blue × Edgescote Purple	13	4	6	3	Corroborative in degree of the 1:1 ratio.
8/20. Majestic × Edgescote Purple.	28	16	8 } 11 }	1	3R. seedlings in 1921 were not planted in 1922. Equality S:R would be expected.
14/22. Majestic × Keston Kidney	15	8	7	—	Approximation to equality in the first result.
14/21. „ „	6	3	1	2	
10/20. Majestic × 30/16, 47	16	9	6	1	Reciprocal of 17/20 where 4S:5R.

Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
15/20. Favourite \times 30/16, 47	25	17	7	1	An excess of susceptibles.
7/21. Majestic \times President	12	10	1	1	Reciprocals of 6/21, 13/22. Unsatisfactory from the large number of poor plants. An equality ratio would be looked for.
7/22. " "	30	8	10	12	
4/25. Golden Wonder \times Edgecote Purple	25	4	20	1	An excess of immunes.

Dealing with these results the sum totals do not give any close approximation to equality among the mixed progenies, 174 seedlings showing 82S:70R and twenty-two plants, 'too poor'; equality would mean 76S:76R. On the other hand a 9:7 ratio would fit slightly better with 85.5S:66.5R, but would not explain the two divergent results met with in family 15/20 Favourite \times 30/16, 47, which gave 17S:7R and 4/25 Golden Wonder \times Edgecote Purple which gave 4S:20R. There are indications in some of the families of an equality ratio however, but the group is rather less amenable to explanation, wide fluctuations being seen particularly in the Majestic \times President results, where, as in the reciprocal, figures were not satisfactory.

Perhaps it is cogent here to recall the results obtained by Salaman and Lesley with Golden Wonder \times Leinster Wonder, two immune varieties. The figures given in the Journal of Genetics, 9S:18R were no doubt the final ones for the test. This result was in marked contrast to the other of the same type, viz. Kerr's Pink \times Champion 2 already commented upon. The Golden Wonder \times Leinster Wonder figures were taken to represent a normal 1S:3R heterozygous segregation, Leinster Wonder by selfing having shown itself to be a normal heterozygote giving 1S:3R. On this assumption my 4S:20R result with Golden Wonder \times Edgecote Purple is unexpected. Unfortunately Golden Wonder does not lend itself to a direct test of its own blood seedlings. I gather it is a variety which is regarded as a 'carrier' of virus troubles, but whether this may affect the seedling quality in relation to reaction to wart disease is doubtful.

It must be recalled that seedlings of 1925 families tested in 1926 did not undergo a second year trial if found resistant, for owing to the heavy disease attack in that year a further trial was deemed unnecessary.

Salaman and Lesley's tables in this class record the following crosses:

Arran Rose \times Sharpe's Victor	16	4	11	1	An excess of immunes.
Snowdrop \times Myatt's Ashleaf	25	16	9	—	Divergent results are seen in two crossings, but totalling 80 seedlings, 41S:39R, an equality ratio is assumed.
" "	32	16	16	—	
" "	25	9	14	2	A 1:1 ratio is assumed.
Edzell Blue \times Myatt's Ashleaf	44	21	23	—	

In this group of experiments with Myatt's Ashleaf, a straightforward result seems to be indicated between Edzell Blue, segregating 1S:3R when selfed and a pure susceptible Myatt's Ashleaf, and also between Snowdrop segregating 1S:3R (see families 3/21, 16/22, 5/24, 11/25, in my Table, immune \times immune) and Myatt's Ashleaf. Previously, on selfing Myatt's Ashleaf, a ratio of 9 susceptibles to 7 immunes was found, and in the cross Myatt's Ashleaf \times Edgecote Purple and the reciprocal, aggregated totals showed 122S:24R, upon which a ratio of 3:1 was based, the conclusion being reached that Myatt's Ashleaf was in reality an immune variety, immunity being inhibited by one or more factors.

Some comment is necessary upon the first cross, namely, Arran Rose \times Sharpe's Victor. In the progeny there is an excess of immunes, yet not so striking as with Golden Wonder \times Edgecote Purple. According to Salaman and Lesley, Sharpe's Victor is a very highly resistant susceptible variety; indeed, the result is used as a basis for assuming that susceptible varieties may offer varying degrees of resistance possibly correlated with their genotypic constitution. Edgecote Purple, on the other hand, may be regarded as a typical susceptible variety, yet the result of its crossing with a probably normal heterozygous immune, Golden Wonder, gave in 1926 a much higher proportion of immune seedlings. No sign of the reaction of the high degree of resistance attributed to the variety Sharpe's Victor is seen in the all susceptible progeny resulting from the cross Eclipse \times Sharpe's Victor; admittedly the number of seedlings tested was small.

The tests in this class, immune \times susceptible carried through by Lunden and Jørstad, of which there were six separate crossings, but with five having the same male parent, viz. Centifolia, an equality ratio was found in five of the tests, and possibly in the sixth. See their Table. An alternative ratio of 5:3 was submitted in one result, but susceptibles were in excess, not immunes as in my family 4/25. The variety Golden Lass was the immune parent used in this particular cross; whether it is synonymous with our Golden Wonder is not known.

CLASS VI. SUSCEPTIBLE \times IMMUNE.

Of crosses made in this manner Salaman and Lesley record but one, and for their purpose aggregate the figures with the reciprocal crossing.

Variety.	No. of seedlings.	S.	R.	Too poor.	Remarks.
Edgecote Purple \times Edzell Blue	28	12	16	—	A total of 93 seedlings, 45S:48R an equality ratio assumed.
Edzell Blue \times Edgecote Purple	27	16	11	—	
" "	38	17	21	—	

In this group of trials my results are as follows:

5/17. Macpherson \times Defiance	7	7	0	—	Macpherson is probably synonymous with British Queen.
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This was one of the results which led me to suggest the dominance of susceptibility, a conclusion subsequently found untenable.

Variety.	No. of seedlings.	S.R.	Too poor.	Remarks.
10/17. Macpherson × Leinster Wonder	3	1	2	—
2/20. Eclipse × Shamrock	10	5	2	3
3/20. Eclipse × Majestic	19	9	9	1
12/20. Up-to-date × Shamrock	8	3	5	—
13/20. Up-to-date × Majestic	5	3	1	1
17/20. 30/16, 47 × Majestic	9	4	5	—
1/21. Midlothian Early × Majestic	5	1	3	1
2/21. Sharpe's Express × Majestic	5	2	3	—
6/21. President × Majestic	11	7	1	3
13/22. " "	28	14	9	5

Other evidence points to President being a pure susceptible and Majestic a heterozygous immune.

8/21. British Queen × Majestic	10	6	2	2	Aggregating 17S : 17R.
9/22. " "	29	11	15	3	
11/21. King Edward × Majestic	13	7	6	—	Evidence for equality.

The progeny of this cross was the subject of a short paper in the 'Journal of Genetics', vol. xiv, 1924, entitled 'Inheritance of the colour-pattern of King Edward potato'. There were grown ninety-two tuber-bearing seedlings, and of these forty-six produced white tubers, and forty-six carried tubers similar to the King Edward. In the test for wart disease, of thirteen seedlings, seven were warted and six immune. Of the six immune there were individuals with the splashed tubers of the King Edward pattern, and which cropped reasonably well. From this evidence it would appear possible to produce an immune King Edward type of potato. The year the cross was made (1920) was the only occasion flowers were observed on the King Edward variety at Merton, Surrey, and then but a few on one plant only.

3/22. May Queen × Flourball	8	4	2	2	May Queen is probably pure susceptible. Early × late varieties.
1/25. May Queen × Majestic	12	3	9	—	Early × mid-season varieties.
2/23. Epicure × Majestic	9	1	8	—	" " " "

It may be no more than a coincidence that the figures from susceptible \times immune varieties, where both susceptible and immune seedlings have been included in the progenies in the above table of my results, give 184 seedlings, 81S:82R and twenty-one 'too poor', a very close approximation to equality; there are outstanding deviations individually, of which perhaps the last, Epicure \times Majestic, is a good example. Here Epicure is an early and Majestic a mid-season variety; the full onslaught of the disease may not have been felt in what possibly may have been a relatively early maturing progeny. It is always wearisome to explain away something in favour of something else, but the same argument is applicable to Salaman and Lesley's Arran Rose \times Sharpe's Victor result.

In the same class of crossings in Lunden and Jørstad's experiments, three show an equality result, one an alternative 1:1 or 3:5 ratio, and two are given as indicating a 3:5 ratio, one immune parent is involved in the equality results, and the other immune parent gives results of a differing order.

For the purposes of record and comparison, a remodelled tabulation of the results of Lunden and Jørstad is given below.

Lunden and Jørstad. 1934.

Immune, selfed, segregating S:R		Immune \times Immune, segregating S:R	
Jubel	1:3	Abundance \times Jubel	1:3
Hindenburg	1:3	¹ Hjelvik \times Jubel	1:3
Pepo	1:3	Edzell Blue \times Jubel	1:7
Pernassia	1:3	Dukker \times Jubel	3:13
Seydlitz	1:3	Kerr's Pink \times Jubel	1:7
Flourball	1:3	Kerr's Pink \times Jubel	1:15
Hindenburg \times Centifolia. R. seed-		Golden Lass \times Flourball	1:3
ling	1:3	Irish Cobbler \times Jubel, all resistant	3:5
"	1:3		
"	1:3		
Tannenburg	1:3		
Immune \times Susceptible, segregating S:R		Susceptible \times Immune, segregating S:R	
Hindenburg \times Centifolia	1:1	Sagerud \times Jubel	3:5
King George \times Centifolia	1:1	Louis Botha \times Jubel	1:1
Golden Lass \times Centifolia	1:1	Early Puritan \times Jubel	3:5
Dukker \times Centifolia	5:3	Marius \times Hindenburg	1:1
Jubel \times Centifolia	1:1	Early Puritan \times Hindenburg	1:1
Dukker \times Geheimrath Haas	1:1	Louis Botha \times Hindenburg	1:1
Susceptible, selfed, segregating S:R		Susceptible \times Susceptible, segregating S:R	
Centifolia, all warted.		Sagerud \times Centifolia, all warted	
		Marius \times Centifolia, all warted	
		Louis Botha \times Centifolia, all warted	
		Early Puritan \times Centifolia, all warted	

¹ syn. with Abundance.

CONCLUSION.

The bulk of the evidence produced seems to be in favour of a simplification of the problem of wart disease inheritance in potatoes rather than the multiplication of immunity factors and others for inhibiting them, each variety of potato being a law unto itself. Our modern commercial varieties must be the outcome of considerable inbreeding, and many are probably closely related, certainly often the product of a naturally set berry, taken haphazard, if one can rely upon hearsay. I am loth to make exceptions for single results or to look for complexity where it is possible that none may exist. Admittedly the Ormskirk trials were excellent, but they had the limitations normally associated with such experiments when dependent on external conditions not under control, the seasonal and soil conditions. It is recognized on all sides that an efficient laboratory controlled technique for seedling infection is desirable, and for that reason the first results of the kind from Lunden and Jørstad are particularly valuable.

SUMMARY.

1. The results of a series of wart-disease trials relative to varieties of potatoes and made at Ormskirk during the period 1918-27 are tabulated.
2. Suggestions are made as to the applicability of certain genetic ratios and comparisons made with other records, but factorial schemes for the genetic constitution of potato varieties are not put forward.

My sincere thanks are due to Mr. H. Bryan, Superintendent of the Ormskirk trials for his help and willing co-operation in securing the records of the potato seedlings submitted to his care.

POSTSCRIPT.

Since going to press a paper by W. Black has appeared in the 'Journal of Genetics', xxx, 127, 1935. In it a simplified factorial notation has been adopted for various varieties and seedlings which have been the subject of investigation.

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The Anatomy of Amaryllidaceous Leaves.

I. Stomatal Distribution in Haemanthus and Brunsvigia.

BY

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With Plate XI and eleven Figures in the Text.

CONTENTS.

	PAGE
I. INTRODUCTION	493
II. METHOD	494
III. NOTES ON MATERIAL USED	494
<i>Haemanthus coccineus</i> , L.	494
<i>Haemanthus rotundifolius</i> , Gawl.	494
<i>Brunsvigia gigantea</i> , Heist.	494
IV. RESULTS OBTAINED	496
<i>a. H. coccineus</i> , L.	496
<i>b. H. rotundifolius</i> , Gawl.	500
<i>c. B. gigantea</i> , Heist.	503
V. SUMMARY	504
LITERATURE CITED	505

I. INTRODUCTION.

THE interesting results obtained by Salisbury (4) when considering variations in stomatal distribution (frequencies) over the individual leaf made it desirable to extend this work to other leaf types. A study has been made of the interaction of stomatal index (the proportion of epidermal units converted into stomata) and the expansion factor (of the ordinary epidermal cells) in determining stomatal frequencies. An attempt has been made to correlate these variations with the variations in water supply during growth. Where epidermal papillae are present, their mature structure has been described and their distribution considered in the same way as for the stomata, that is, the papilla frequencies and indices have been determined.

Salisbury (4) examined the epidermis of some elongated monocotyle-

donous leaves and found that the stomatal frequencies, i.e. the number of stomata per unit area of leaf surface, increase from the base to the apex of the leaf, and from the mid-rib to the margin. He found that these gradients are of 'widespread occurrence', and are also modified under certain conditions. Thus the leaves of *Carex sylvatica* show a rapid increase in the frequency values for a short distance from the base of the leaf, followed by a gradual fall towards the apex. Salisbury (4) expressed the numerical relationship of the stomata to epidermal cells by the formula

$$I = \frac{S}{E + S} \times 100,$$

where I = the stomatal index, S = the number of stomata per unit area, and E = the number of epidermal cells for the same unit area. He worked mainly with comparatively small linear leaves, and found that the index values for a given species are more constant than the frequencies. In the present investigation, large leaves of simple outline have been used, in which there is an even distribution of sub-epidermal mesophyll cells, giving a continuity of stomatal distribution uninterrupted by sub-epidermal bands of sclerenchyma.

II. METHOD.

Counts were made of the epidermal constituents (stomata, papillae if present, and ordinary epidermal cells) at numerous points on the leaf surface, but chiefly along the margins and the mid-rib of the leaf. These counts were made with a Leitz Wetzlar projection apparatus by means of which an image of the piece of epidermis under observation was projected on to a sheet of squared paper. Each cell was indicated by marking the position of its nucleus and at the same time a certain portion of the field was drawn accurately so that the variations in the sizes of the cells could be obtained by simple readings. For these broad leaves a large epidermal area of $2\frac{1}{4}$ sq. mm. was used, as this minimizes errors in considering cells that do not come entirely within the area examined.

III. NOTES ON MATERIAL USED.

Haemanthus coccineus, L.

Haemanthus rotundifolius, Gawl.

Brunsvigia gigantea, Heist.

Haemanthus coccineus and *H. rotundifolius* are widely distributed in the south western region of South Africa and both occur on the Cape Peninsula, the former usually growing in rock fissures high up on the mountain slopes, and the latter being found in sandy places both on the plains and in level portions of mountainous regions.

Bolus (1) and Levyns (2) record only one species, *H. coccineus*, on the Cape Peninsula, though the latter writer adds 'two distinct plants seem to be included under this species'.

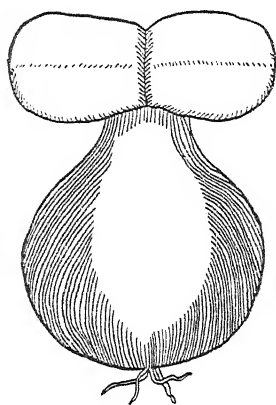
Before agriculture and building had destroyed the native vegetation, *H. rotundifolius* was probably common around Table Bay and sailors coming ashore for fresh water would frequently meet with it. This is the explanation suggested for the early date (1644) of the first description of this plant. It is still plentiful in undisturbed areas such as the Silver-tree forests of Kirstenbosch.

Apart from the usual taxonomic characters used for the separation of these two species, the roughness of the papillae on the upper surface of the leaf of *H. rotundifolius* distinguishes it at once from *H. coccineus*, the upper epidermis of which is smooth. It is only necessary to rub the tip of the finger lightly over the upper epidermis to distinguish easily the two species.

Of the two species of *Haemanthus* which were used in this investigation, *H. rotundifolius* is of considerable historical interest, as it is one of the small group of plants observed at the cape by Justus Heurnius and subsequently figured and described by J. B. à Stapel (5) in 1644. In the above work, *H. rotundifolius*, Gawl.¹ is shown in winter condition, with two large, suborbicular leaves horizontally extended. Text-fig. 1 is a copy of the illustration and should be compared with Pl. XI, Fig. 4.

Both species of *Haemanthus* used in this investigation have scaly bulbs with many thick bifarious tunics. All the bulb scales are leaf bases, each scale showing a brown apex where the green portion of the lamina has withered away. A comparison of Pl. XI, Figs. 1 and 3 shows that the apex of the bulb in *H. rotundifolius* is proportionally much broader than in *H. coccineus*, a feature correlated with the very broad leaf base in the former species. The bulb of *Brunsvigia gigantea* is truncated in contrast to the scaly one of *Haemanthus*. A large bulb produces six lingulate leaves, which lie more or less adpressed to the ground when mature, though the degree to which this occurs is determined largely by the surrounding vegetation. The edges of the leaves are minutely ciliated, the cilia being red on the young leaves and turning brown on the older leaves.

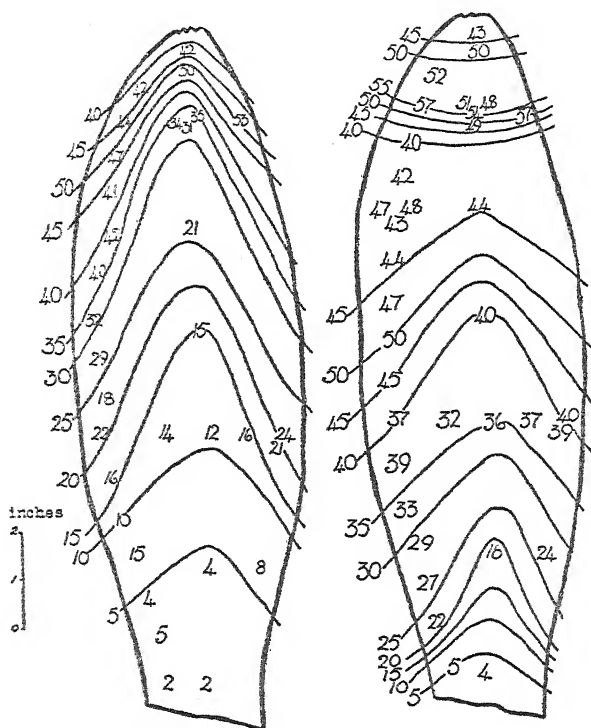
A plant grown in Bedford College greenhouse was found to exhibit considerable movements of its leaves during the growing period. The young leaves do not all move at the same rate, some of them performing a



TEXT-FIG. 1. Copy of drawing of *H. rotundifolius* published by J. B. à Stapel in 1644.

¹ Linnaeus, however, identified the figure as *H. coccineus* L. (Linn. Fl. Cap. p. 4, 1759).

rise to the vertical and a fall to the horizontal once in every 48 hours and the others once in every 24 hours. Thus it frequently happens that some



TEXT-FIG. 2.

TEXT-FIG. 3.

TEXT-FIGS. 2-3. *H. coccineus*. Lines drawn through points with the same stomatal frequency values. 2. Lower surface. 3. Upper surface.

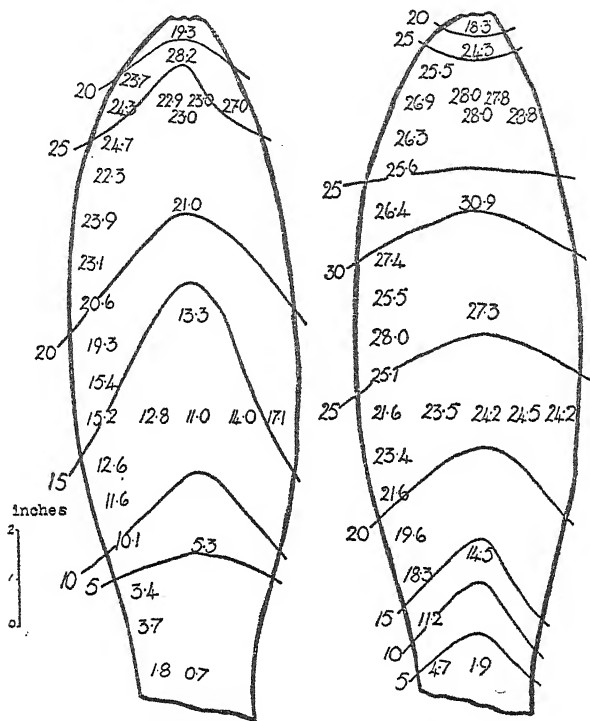
of the leaves are rising while others are falling or some of the leaves are vertical while others are horizontal. Finally, at the end of the growing period movement slows up and finally ceases, all the leaves lying flat on the ground.

IV. RESULTS OBTAINED.

(a) *Haemanthus coccineus*.

The first leaf of *H. coccineus* examined was $14\frac{1}{4}$ inches long, with a maximum width of 5 inches, and was grown from a bulb in the greenhouse. The leaf was fixed in strong alcohol, and specimens of the epidermis were taken from a number of places on the leaf surfaces, both upper and lower sides being examined. For each area, the stomatal frequency (number of stomata per unit area) and the stomatal index (percentage of epidermal units converted into stomata) were determined and the results expressed as diagrams.

Text-figs. 2 and 3 give the results of the stomatal frequency. The figures placed on the leaf-surface in Text-fig. 3 represent the actual values obtained in these areas. Lines were drawn through areas of approximately



TEXT-FIG. 4.

TEXT-FIG. 5.

TEXT-FIGS. 4-5. *H. coccineus*. Lines drawn through points with the same stomatal index values. 4. Lower surface. 5. Upper surface.

equal frequency, the values of these lines are given *outside* the left margin of the leaf. A similar treatment was accorded the lower surface and the results are shown in Text-fig. 2.

The stomatal indices were also obtained for the upper and lower surfaces of the same leaf and the results expressed in a similar way in Text-figs. 4 and 5.

The stomatal frequency values, for the margin and median line of both upper and lower surfaces of the mature leaf, increase from the base to near the apex where there is a slight fall, as shown in Text-fig. 6. For the upper surface a marked irregularity occurs in the apical half of the leaf, the values falling and then rising to a slightly higher point. This irregularity is not found on the lower surface. The stomatal frequency gradient increases from the median line of the leaf to the margin as shown in Text-fig. 8.

The stomatal index values, for the margin and median line of both surfaces of the leaf, increase from the base to near the apex, where there is a slight fall (Text-fig. 7). There is also a small increase in stomatal index values from the median line to the margin. The stomatal index values tend to be more constant than those of the frequencies, as is well seen by comparing the gradients, which are much steeper for the frequencies.

At least two factors determine the stomatal frequency gradients, these are, the index variations and the variations in the expansion of the epidermal cells. The importance of this second factor is shown by the failure of the frequency curves to correspond exactly with those of the indices. If drawings are made of successive pieces of epidermis, of equal area, at definite points along a line proceeding from the base to the apex of the leaf, then the variations in the size of the individual cells can be obtained. Thus at the base of the leaf the cells are greatly extended longitudinally in *H. coccineus*. Proceeding towards the apex the cells become smaller, and at the apex numerous very small cells are present among the larger ones.

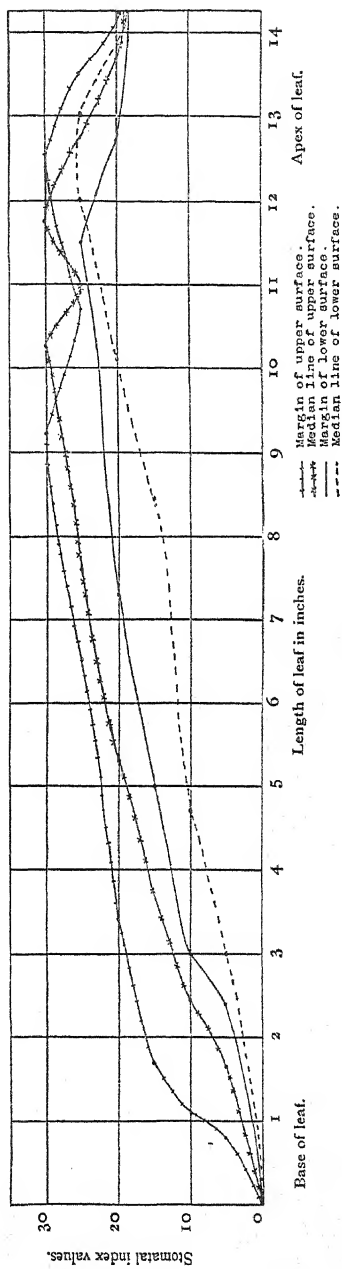
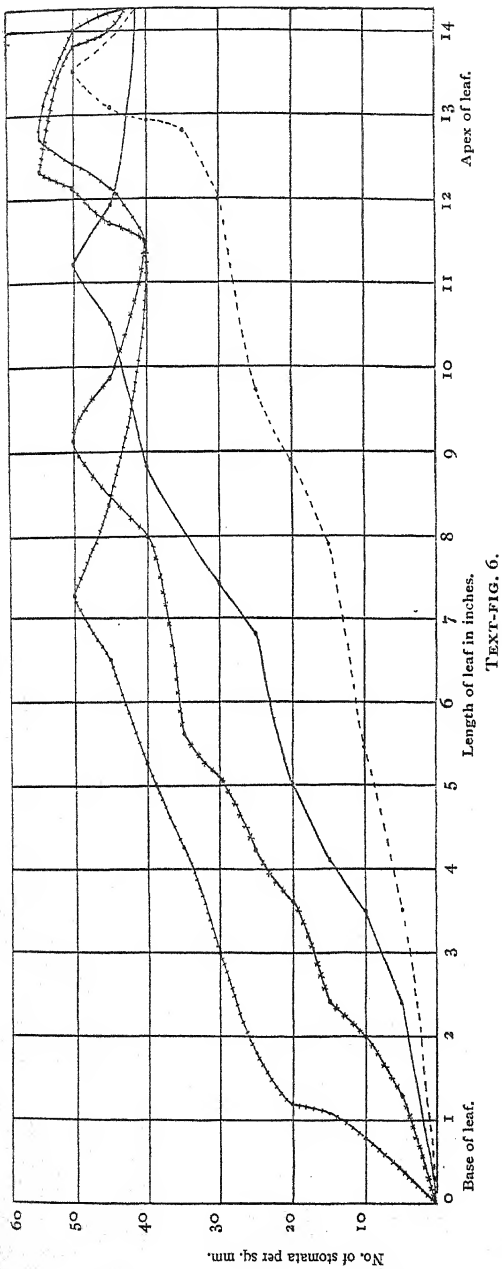
Corresponding pieces of epidermis taken from the upper and lower surfaces show that the epidermal cells of the lower epidermis have undergone greater expansion than those of the upper, but the difference is not so marked as in the leaves to be described later.

The variations in the degree of expansion of the epidermal cells is a direct outcome of the variations in the amount of water supplied to the young extending portions of the leaf, mainly in the region of auxesis, during growth. The leaf-base and the apex of the leaf-limb are the first formed parts of the leaf. Increase in the length of the leaf-limb is due to the activity of the tissues at the base of the leaf-limb. The leaf-base remains within the bulb and acts as a storage organ and so is only of indirect importance in the present consideration. When the tissues of the apex of the young leaf are maturing (auxesis period) the final dimensions of the cells depends on the water supply at that period.

The apex is dependent for its water supply upon the amount of fully differentiated and lignified xylem in the vascular bundles passing through the meristematic zone at the leaf-base. At this stage only the protoxylem is lignified and capable of water conduction, the metaxylem consisting of immature tracheids with considerable cell contents. Thus it is obvious that the amount of water passing to the leaf apex must be small, resulting in the production of comparatively small cells.

During the growing period the metaxylem passing through the base of the leaf-limb gradually becomes lignified and the vascular tissue also increases in amount in the individual bundles, and as a result the successive intercalary pieces added to the leaf-limb each have a better water supply than the previous ones, causing a greater expansion of the epidermal cells.

The last-formed portions at the base of the leaf-limb were formed



FIGS. 6-7. *H. coccineus*. 6. Stomatal frequencies for upper and lower surfaces. 7. Stomatal indices for upper and lower surfaces.

when the water supply was at its maximum, and so the epidermal cells are very greatly extended longitudinally. This great length combined with the low index value results in a very low frequency value.

In considering the correlation of water relations and frequency variations over the individual leaf Salisbury (4, p. 24) 'states that the low rate of water intake by portions of the leaf remote from the water supply favours high stomatal frequencies'. As already explained, two factors have to be considered here. Firstly, whether the region under consideration is mature or not, and secondly, the nature of the conducting tissues. In considering a matured portion of the leaf it is essential to know what was the nature of the water supply when the tissues were expanding. The results for *H. coccineus* show that, although the apex of the immature leaf is comparatively near the water supply, the expansion factor is at a minimum owing to the small number of actively conducting xylem elements.

(b) *Haemanthus rotundifolius*.

Two leaves of *H. rotundifolius* were examined, a leaf 9 cm. long and 12.3 cm. wide from a small bulb and a leaf 28.5 cm. long and 28 cm. wide from a large bulb. Both leaves examined were collected in South Africa from plants in the wild state.

The leaf, which lies adpressed to the ground, has the papillae confined to the upper surface. Papillae are only present on the exposed green portion of the leaf and their occurrence ceases abruptly where the leaf enters the bulb, as shown in Table I.

The distribution of the papillae is very irregular, especially on the large leaves, but on the whole the highest papilla frequencies are found near the base of the leaf. The papilla indices (i.e. the proportion of epidermal units converted into papillae), show similar variations to those of the papilla frequencies.

TABLE I.

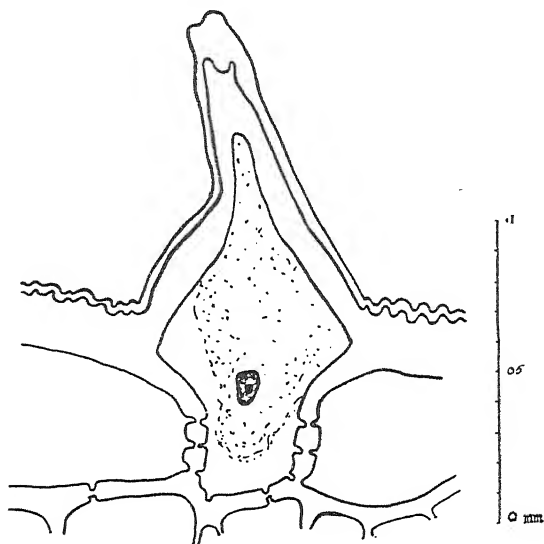
Papilla frequencies of a Large Leaf of H. rotundifolius.

(Per sq. mm. of leaf surface.)

	Base of leaf.													Apex of leaf.					
Margin of leaf	0	0	0	23	24	19	13	12	22	13	17	20	17	21	19	18	13	19	15

The papillae are roughly conical with furrowed and ridged sides. The thick wall of the projecting conical part of the papilla consists of a comparatively thin outer cuticularized layer upon a thick inner cellulose wall. The narrowed base of the papilla which is sunk in the epidermis, consists of a somewhat thinner cellulose wall which is deeply pitted, the pits connecting with the neighbouring epidermal cells (Text-fig. 8). The apex of

the papilla is mucilaginous, and numerous foreign particles are frequently found adhering to it. The mucilage appears to be derived from the chemical alteration of the cuticular portion of the papilla wall at the apex of the papilla. The mucilage forms a cap over the apex of the papilla, but



TEXT-FIG. 8. *H. rotundifolius*. Longitudinal section of a papilla.

the mucilage is not evenly distributed, probably due to the fact that a certain amount of it has been washed away by rain. The papilla contains a well-developed protoplast with a large nucleus. The function of the papilla is discussed in the account of similar structures found on the leaves of *Brunsvigia gigantea*.

The stomatal frequencies for the upper surface of *H. rotundifolius* are very low at the base of the leaf in the yellow portion just within the bulb, and the values increase rapidly at the base of the exposed portion, that is, the base of the free green leaf. There is then a gradual increase towards the apex of the leaf, both along the margin and the median line of the leaf, although the gradients are not so well marked as in *H. coccineus* (Table II). The stomatal frequency gradient is much steeper for the lower surface than for the upper in the large leaf examined.

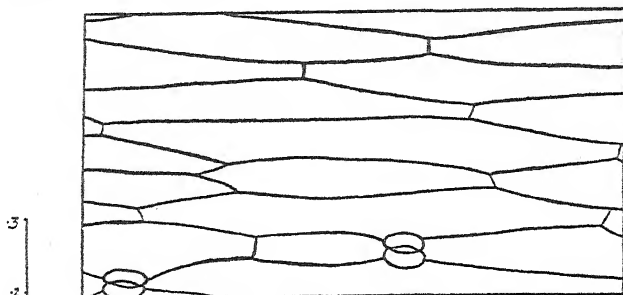
TABLE II.

Stomatal Frequencies for a Large Leaf of H. rotundifolius.

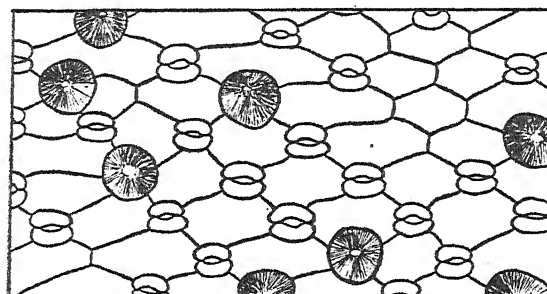
(Per. sq. mm. of leaf surface.)

	Base of leaf.										Apex of leaf.			
Upper surface.														
Medium line	1	1	2	33	46	51	50	42	43	39	43	48	47	49
Lower surface.														
Median line	0	2	2	6	13	10	12	16	20	20	22	22	28	28

The stomatal indices, for the small leaf examined, vary between 25 and 30 for the upper surface and between 10 and 20 for the lower. The gradients are similar in form to those of the frequencies, but they are not so steep owing to the influence of the expansion factor. The variations in the



TEXT-FIG. 9.



TEXT-FIG. 10.

TEXT-FIGS. 9-10. *H. rotundifolius*. Epidermis from the base of the leaf for the lower and upper surfaces respectively.

degree of expansion of the epidermal cells are most marked on the lower surface. Large, longitudinally extended cells are found near the base of the leaf, Text-fig. 9, and there is a decrease in size towards the apex where the individual cells average only half the length of these. The epidermal cells of the upper surface show only a slight expansion gradient from the base to the apex of the leaf.

The contrast in the stomatal frequency between the lower and upper surfaces shown in Text-figs. 9 and 10 is partly due to the lower index values for the lower surface compared with the upper (11.3 for the lower and 24.5 for the upper) and partly to the much greater expansion of the epidermal cells on the lower surface, causing the wider spacing of the stomata.

In comparing the leaves of *H. coccineus* and *H. rotundifolius* it is found that the stomatal frequencies for the lower surface of *H. coccineus* range from 0 to 40 per sq. mm. of leaf surface and for *H. rotundifolius* the

corresponding figures are 0 to 20. The corresponding index range for both leaves is 0 to 20. Examination of the epidermal cells of the lower surface of both leaves shows that there has been greater expansion of the epidermal cells for the leaf of *H. rotundifolius*. Thus the higher stomatal frequency values for the lower surface of *H. coccineus* are due to the smaller amount of expansion of the epidermal cells compared with *H. rotundifolius*. The great expansion of the epidermal cells and the resultant wide spacing of the stomata in *H. rotundifolius* can be correlated with the prostrate habit (Pl. XI, Fig. 4). Owing to the water that must collect on the lower surface this leaf is practically in the condition of a floating aquatic, the stomata being rendered partly or completely functionless. In *H. coccineus*, with erect habit, there is no such contrast between upper and lower surfaces in the physiological conditions or in the stomatal relationships.

(c) *Brunsvigia gigantea*.

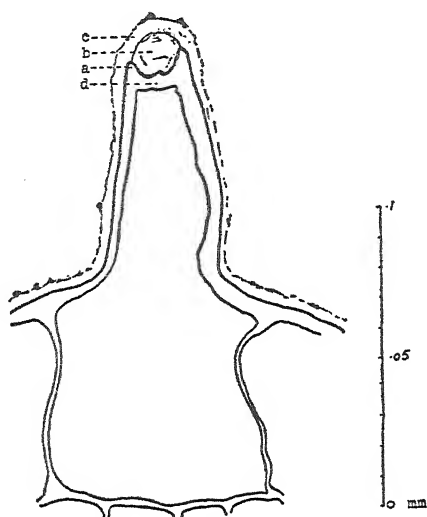
The upper surface of *B. gigantea* has numerous simple papillae. The papilla frequencies vary between 16 and 26 per sq. mm. of leaf surface, but no definite gradients from base to apex were obtained. The projecting portion of the papilla is cylindrical with a rounded apex. The base of the papilla, which is embedded in the epidermis, is thin-walled and has no pits. In longitudinal section the wall of the free portion of the papilla is seen to be thick, a comparatively thin outer portion of which is cuticularized. The apex of the papilla consists of a mucilaginous cap, *c* in Text-fig. 11, within which is an internal projection, *b*. The inner cellulose portion of the wall forms a chamber at the apex of the papilla by a circular outgrowth *a*, in Text-fig. 11. The upper chamber is cut off by a thick cellulose wall, *d*, in Text-fig. 11, in which there is no pore to connect with the lumen of the papilla. The papilla has a well-developed protoplast, with a large nucleus which is most often found in the upper portion.

The structure of the papillae of *H. rotundifolius* and *B. gigantea* suggests that they have a specialized function. Mr. Garside suggests that the papillae may function when the leaf is very young, secreting mucilage and protecting it in some measure from drought. Afterwards, when the epidermis is exposed to the air, the mucilage dries up at the tips of the papillae. He also suggests in this connexion that the wall (*d*) of Text-fig. 11 may be formed as the cell ages and becomes mature.

I would suggest that these papillae may function in another way, that is by absorption of water from the atmosphere. This suggestion is based on an examination of a young leaf of *B. gigantea* growing under greenhouse conditions, and comparison of these papillae with those described by Marloth (3). Marloth found water-absorbing hairs on the leaves of several South African plants, especially those growing under semi-arid conditions. He describes water-absorbing hairs of *Eriospermum pustulatum* Marl.

which are similar in structure to the papillae of *H. rotundifolius*, having thick outer walls and pitted bases.

The stomatal frequency values increase from the base to the apex of the leaf and from the median line to the margins for both surfaces, but



TEXT-FIG. 11. *Brunsvigia gigantea*. Papilla in longitudinal section. *a* = ring of cellulose, *b* = mucilage projection, *c* = apical cap of altered cuticle, *d* = cellulose wall at apex of papilla.

there is no fall at the apex for the upper surface as in *H. coccineus*. The stomatal frequency values are higher for the upper surface than for the lower. The index values are more constant than the frequency values, but the gradients for both have the same general form.

There is a well-marked expansion gradient for the lower surface, the epidermal cells decreasing in size from the base to the apex of the leaf. The epidermal cells of the upper surface are much smaller than those of the lower at corresponding points, and the upper surface shows no expansion gradient. Thus this contrast between the upper and lower surfaces is characteristic of the two prostrate leaves examined, that is for *H. rotundifolius* and *B. gigantea*.

V. SUMMARY.

1. The large, broad, amaryllidaceous leaves considered in this investigation show similar stomatal frequency-gradients to those described by Salisbury for some narrow leaves. That is, a general increase in frequency values from the base to the apex, and from the median line to the margin of the leaf.

2. The stomatal index values are more constant than those of the frequencies for the leaves of the three plants examined. In addition to the

stomatal index value, a second factor, the expansion of the epidermal cells, plays an important part in determining the frequency variations. There is generally a decrease in the size of the epidermal cells from the base to the apex and from the median line to the margin of the leaf. In the prostrate leaves examined there is a much greater expansion of the lower epidermis than of the upper. The expansion gradient is determined by the variations in the water supply to the successive intercalary pieces added to the base of the leaf-limb when these pieces are undergoing auxesis.

3. Epidermal papillae are described for *H. rotundifolius* and *B. gigantea*, their distribution is given and the nature of their function is suggested.

4. Movements of the young leaves of *B. gigantea* are described when growing under greenhouse conditions.

In conclusion, I wish to thank Mr. Garside of Bedford College for material collected in South Africa and for historical notes on these plants. I also wish to thank him for his valuable help, criticism, and advice in the preparation of this paper.

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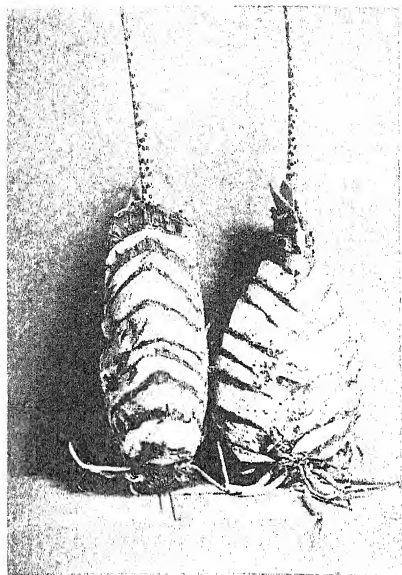
EXPLANATION OF PLATE XI.

Illustrating Miss Wicks' paper on 'The Anatomy of Amaryllidaceous Leaves. I. Stomatal Distribution in *Haemanthus* and *Brunsvigia*'.

Figs. 1-2. *Haemanthus coccineus*. 1. Bulbs and inflorescence stalks obtained from Stellenbosch Mountains, S. Africa. 2. A plant with foliage leaves growing in Bedford College greenhouse.

Figs. 3-4. *Haemanthus rotundifolius*. 3. Bulbs and inflorescence stalks obtained from Stellenbosch Mountains, S. Africa. 4. Leaves in nature, Stellenbosch Flats, S. Africa.

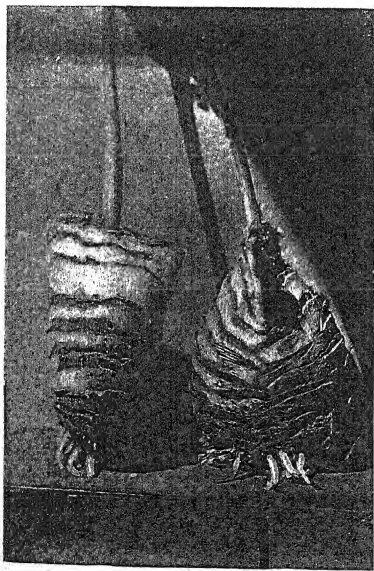
(The Photographs of figures 1, 3, and 4 were taken by Mr. Garside in S. Africa.)



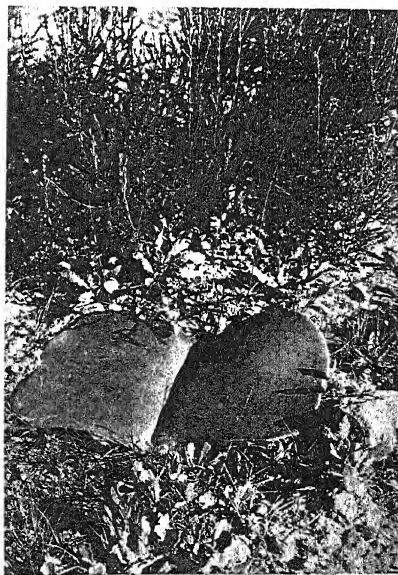
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4

Disarticulation of the Branches in Eucalyptus.

BY

A. J. EWART, D.Sc., F.R.S.

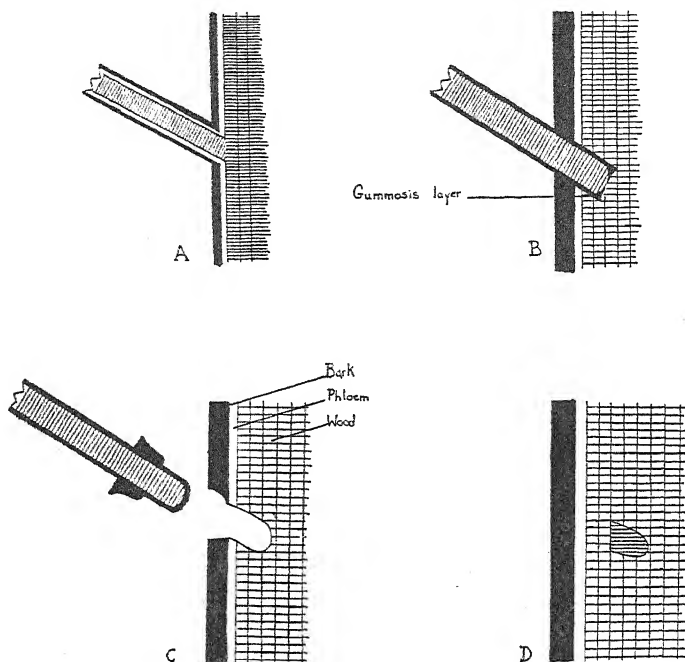
(*Professor of Botany and Plant Physiology at Melbourne University.*)

With Plate XII and one Figure in the Text.

ONE of the needs of forestry in growing marketable timber is to produce trees having a straight cylindrical bole without lateral branches so that the tree when cut and sawn yields a maximum amount of even straight-grained timber free from knots or defects. The suppression of the lateral branches in coniferous trees is, of course, produced by close planting and thinning out the trees as soon as a continuous canopy is produced which shades the lower lateral branches to such an extent that they die. In most climates these dead branches in time soften sufficiently to either break away or to be broken off by the push exerted by the outwardly growing wood of the main trunk, but in a drier climate, like that of a large part of Australia, one of the difficulties in planting coniferous trees is due to the fact that the dead lateral branches persist for an unusual length of time however closely planted the original stand of trees may have been. Such persistent branches not only leave behind knots in the timber of considerable length, but also form points at which the penetration of parasitic fungi is possible while the tree is living. To remove the dead lateral branches by a saw, axe, or bill-hook means considerable expense in large plantations, the cost of which is not always justified.

One of the advantages which many species of Eucalyptus have over conifers lies in the fact that in many cases eucalypts are able to shed their branches just as a deciduous tree is able to shed its leaves, and it is for this reason that many eucalypts such as the Mountain Ash, *E. regnans*, the Red Stringy-bark, *E. macrorrhyncha*, and Messmate, *E. obliqua*, when adult may exhibit stems with the lower 100 ft. or so completely free from any evidence of a branch, although during the growth of the trees, lateral branches may have been formed in abundance from the butt to the top of the tree. This peculiarity may be shown even when the trees are not crowded when young, although naturally in exposed situations the tendency to form persistent side branches usually becomes evident earlier than when the trees are in close formation. The process of disarticulating the branches,

although comparable with the typical mode of disarticulation of deciduous foliage leaves, is carried out in a different manner. The deciduous leaf, strictly speaking, is thrown off before the leaf is quite dead and the formation of an abscission layer at the base of the leaf is simply the last act in the



Eucalyptus macrorrhyncha. Shedding of branches by gummosis. A. Side branch living. B. Side branch two years later. C. Three years later. Branch shed. D. Five years later. Wound healed and cavity filled with new wood.

life of the leaf. The abscission of the leaf is due to the formation of this special layer of cells at its base. In the case of the disarticulating branches the branch dies long before it is thrown off, and this death of the lateral branches may be due either to the high light requirements of the foliage in taller eucalypts or may be due to the top of the tree exercising a greater pull on the water travelling up the main trunk. Some time after the lateral branches have died, the period varying from one to three years, the branches will be found to have come loose, and by working them to and fro they will come away from the main stem, showing a rounded base covered by dark gum and often bringing with them a little sliver of bark, but without its being necessary to break the wood at the base of the branch. The base has, in fact, been eaten away and replaced by a soft layer of gum containing no wood-fibres or organized structural elements. Evidently, therefore, the tree is able to produce and concentrate at the base of the branch a lignase or other enzyme which slowly dissolves the wood at the base of the branch

and converts it into a layer of gum. In natural conditions the branches slowly work free and ultimately fall from the tree aided by the wind and their own weight. The length of time to disarticulate a branch depends mainly on its size. It is rarely that a tree is able to disarticulate a branch more than one inch in thickness at its base, and to disarticulate a branch of this size usually takes at least three years. During this period the disarticulating base of the branch may be left 2 or more inches below the outer surface of the bark with the butt buried $\frac{1}{4}$ to $\frac{3}{4}$ in. in the later layers of wood, the depth depending on the time required to dissolve the base of the branch and upon the rate of growth of the wood on the main trunk. After the branch has fallen it may leave at first a hole in the surface of the wood which ultimately is filled up by the activity of the surrounding cambium, but if the branch is a large one, or if the tree has been damaged by bush-fires, the cambium may grow over the cavity superficially, leaving a gum pocket in the wood. The photograph (Pl. XII, Fig. 1) shows some of the knot holes left in a stem of Red Stringy-bark, *E. macrorrhyncha*, by branches which have just disarticulated. In Pl. XII, Fig. 2, sufficient of the wood has been removed to show the gummy layer at the base of the knot hole. These knot holes, though numerous and close together, would ultimately fill up and leave practically clean uniform timber where they had been. It is difficult to say what stimulus gives rise to the production of this slow-acting lignase enzyme at the base of the branch. No evidence of any such action can be detected while the branches are alive, so that the stimulus for the production of enzyme is apparently due to some reaction between the dead wood of the branch and the living wood or cambium of the tree. Various attempts have been made to extract an enzyme from the bases of disarticulating branches which would be capable of dissolving wood outside the plant. No success has, however, been obtained, possibly because the enzyme is either very small in amount or because its action is so slow as to make test-tube experiments difficult.

L. R. Kerr¹ showed that in the ligno-tubers formed by many seedling eucalypts as temporary storage organs, new shoots were able to develop deep in the tissue of the ligno-tuber, and that these shoots have a digestive cap which dissolves the tissues outside, as in the case of the endogenous development of roots. In this case, also, no lignase enzyme could be detected by making extracts from ligno-tubers which were forming young shoots freely. Nevertheless, the ligno-tubers contain other enzymes, for instance, at temperatures below 5°C. the starch of the ligno-tubers is converted into soluble starch and then into sugar, whereas at 20°C. the cells of the ligno-tubers in a few days become again packed with starch grains. The fact, however, is well known that the hyphae of many wood-destroying fungi can penetrate the walls of wood fibres and wood vessels

¹ Proceedings of the Royal Society of Victoria, 1925, vol. xxxvii, p. 79.

either by dissolving the lignin or by dissolving the cellulose basis in which the lignin is deposited. It seems probable that the nature of the enzyme in this case is similar to that responsible for the disarticulation of eucalypt branches. If it were a case of dissolving the cellulose basis of the wood one would expect the gum produced to give strong lignin reactions, which does not appear to be the case.

Attempts were made to produce artificial disarticulation by driving pegs of coniferous and eucalyptus wood into the trunks of eucalypts which naturally disarticulate their branches. A large number of experiments of this kind were made on seven species of eucalyptus. The pegs after one and two years showed no signs in any case of becoming loosened, and on withdrawing and examining them no distinct signs of any solution of the wood at the base of the peg where it passed through the cambium layer could be perceived. Apparently it is not possible to produce artificially the conditions set up in a living tree. The problems which remain to be solved are:

1. Is the solution of the base of the branch due to a cytase or a lignase enzyme or to a combination of enzymes?
2. What factors induce the formation and localized action of such enzymes?

The following are lists of species of eucalyptus which disarticulate their branches by gummosis and of those species of eucalyptus in which no disarticulating branches have been found.

Branches disarticulating by gummosis.

E. alpina Lindl.
E. botryoidea Sm.
E. Cambagei Deane and Maiden.
E. cinerea F. v. M.
E. cornuta Labill.
E. diversicolor F. v. M.
E. elaeophora F. v. M.
E. gigantea Hk. f.
E. globulus Labill.
E. goniocalyx F. v. M.
E. grandis (Hill) Maiden.
E. Gunnii Hk. f.
E. hemiphloia F. v. M.
E. Macarthuri Deane and Maiden.
E. macrorrhyncha F. v. M.
E. Maidenii F. v. M.
E. melliodora A. Cunn.
E. Muellieriana Howitt.
E. obliqua L'Herit.
E. punctata D. C.
E. radiata Sieb.
E. regnans F. v. M.
E. rostrata Schlecht.
E. saligna Sm.
E. sideroxylon (Woolls) A. Cunn.
E. tereticornis Sm.
E. viminalis Labill.

Non-disarticulating branches.

E. australiana Baker and Smith.
E. Baueriana Schauer.
E. Behriana F. v. M.
E. calycogona Turcz.
E. cladocalyx F. v. M.
E. dumosa A. Cunn.
E. Gilleni Ewart and Kerr.
E. gracilis F. v. M.
E. gummifera Hochr.
E. incrassata Labill.
E. longifolia Link.
E. odorata Behr.
E. oleosa F. v. M.
E. ovata Labill.
E. piperita Sm.
E. polybractea R. T. B.
E. resinifera Sm.
E. uncinata Labill.
E. viridis R. T. B.
E. vitrea R. T. B.

The eucalypts with non-disarticulating branches are mainly shrubs or trees which branch at a low level, whereas the majority of those with disarticulating branches are tall trees with trunks smooth and unbranched to a considerable height. There are, however, some exceptions. *E. alpina* is a shrubby form, branching from the base, but often shows plenty of disarticulating branches.

EXPLANATION OF PLATE XII.

Illustrating Dr. A. J. Ewart's paper on 'Disarticulation of the Branches in Eucalyptus'.

Figs. 1 and 2. Wood cylinder of *E. macrorrhyncha*, 3 in. diameter, showing temporary knot holes left by disarticulating branches. In Fig. 2 a portion of the wood has been removed to show the bases of two knot holes with dark gum layer.

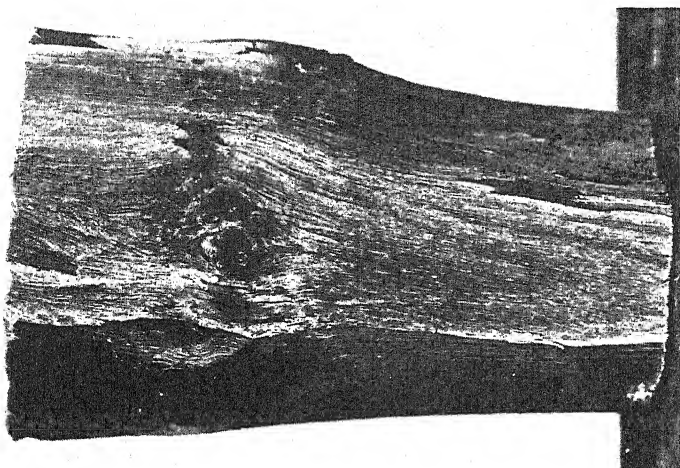


FIG. 2

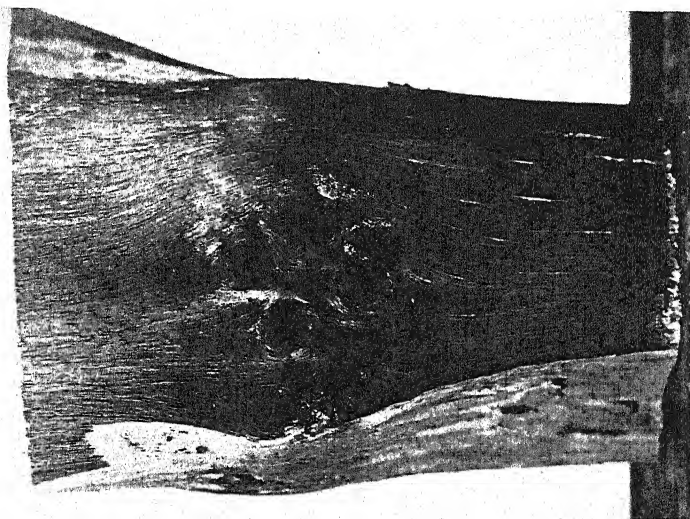


FIG. 1

The Gametophyte of *Phylloglossum Drummondii*.

BY

J. E. HOLLOWAY, D.Sc.

(*University of Otago, N. Z.*)

With six Figures in the Text.

IN 1901 Thomas (6) published a preliminary account of the gametophyte of *Phylloglossum*. This account was based upon ample material collected in the vicinity of Auckland, N.Z., and it gave details with respect to the external features of both the gametophyte and the attached embryo. No figures, however, were given, it evidently being the author's intention to follow up this paper with a more detailed account.

Sampson (5) in 1916 contributed a short note on a poorly preserved gametophyte which was attached to a young sporophyte, this being part of some Australian material which had come into his hands. He gives a figure of this gametophyte as seen in transverse section, but owing to its condition very little information as to its form and structure can be gathered from the figure.

Some years ago the writer visited Auckland for the purpose of searching for the gametophyte of *Phylloglossum*. Likely-looking turves containing sporophytes were taken from a good *Phylloglossum* locality, and from one of these a single healthy mature gametophyte was later dissected out. Publication of the facts so obtained was delayed in the hope that additional material would be found. The writer later paid a second visit to Auckland for this purpose, but without success. Turves containing a large number of mature sporophytes were secured, and were kept in coarse damp sand under greenhouse conditions for two successive seasons. The sporophytes died down to their tubers, came up again the following season, formed cones and shed their spores naturally. Care was taken to ensure that the spores penetrated into the soil. Careful dissection at intervals, however, revealed no gametophytes. The present paper is a description of the single mature specimen gathered in the field.

GENERAL FORM AND STRUCTURE OF THE GAMETOPHYTE.

Thomas refers, among other details, to the following: the general similarity of the gametophyte of *Phylloglossum* to that of *Lycopodium cernuum*; the complete absence of leafy lobes on the crown, as compared

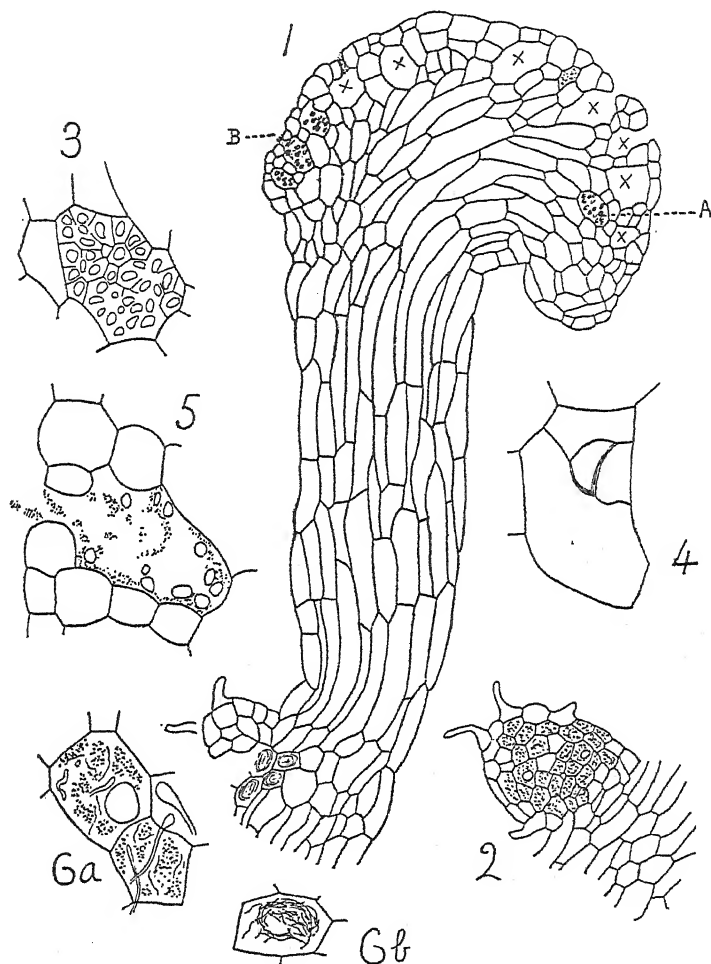
with that of *L. cernuum*; and the fact that as a result of one-sided growth, the crown in *Phylloglossum* is sometimes 'shaped like the head of a horse'. In all these particulars the single gametophyte described below corresponds closely with the description as given by Thomas.

It is about $1\frac{1}{2}$ mm. in height, but is curved below, somewhat after the fashion of the lower part of the letter S. It grew erect, the smooth, round, light-green crown projecting slightly above the soil surface. A large number of antheridia were clearly visible in surface view, embedded all over the crown. Sections showed that no archegonia were present. At the base is a rounded tubercle from which short rhizoids project. The intermediate shaft is well developed. Fig. 1 represents this gametophyte in longitudinal section, median through crown and shaft, but tangential through the tubercle. The latter is shown in median section in Fig. 2, its fungal endophyte being in this figure indicated diagrammatically.

On account of the S-shaped curvature of its basal part it cannot be stated for certain from a study of the sections whether or not the object is here seen in its complete original shape and length. The shaft may be incomplete below (as indeed it might seem to be in Figs. 1 and 2), in which case the tubercle shown is borne laterally on it and is not the original basal tubercle. The writer has found (3) that in three New Zealand species of *Lycopodium* belonging to the *Cernua* section of the genus, namely, *L. cernuum*, *L. laterale*, and *L. ramulosum*, the gametophyte may not only have a much elongated shaft, but may also have one or more laterally placed fungal areas on the shaft in addition to that at the base (see 3, Figs. 38-41, 47, 50-65). Thomas states (6, p. 287) that the shaft in the gametophyte of *Phylloglossum* varies considerably in length, the longest gametophytes observed by him being up to 6 mm. in total length, viz. four times as long as that figured in the present paper. The writer is inclined, however, to regard the gametophyte here described as a complete one, judging from the original notes made when it was first dissected out from the turf.

The cells of the shaft are much elongated, as also are those which run up into the centre of the crown. The meristem which has given rise to the swollen crown is scarcely to be distinguished owing no doubt to the fact that the formation of new tissue with young antheridia had ceased. As can be seen in Fig. 1, however, the youngest antheridia on the crown lie nearest to the top of the shaft, so that the meristem undoubtedly was located here, as Thomas describes, and as is the case in the *L. cernuum* gametophyte. Owing to unevenness in meristematic activity the crown has grown over appreciably to one side. Thomas describes this as taking place in some of the gametophytes examined by him, and adds that when an embryo is present, the upper region of the shaft immediately below the meristem also increases considerably in bulk.

The cells of the tubercle are more or less equi-dimensional in shape and contain the fungal hyphae (Fig. 6, *a* and *b*). Short rhizoids are formed freely on the tubercle. Thomas states that they may also arise from the



FIGS. 1-6. *Phylloglossum Drummondii*, Kunze. Gametophyte. 1. Crown with antheridia and shaft, in median long. sect. Empty antheridia (x). Fungal tubercle in tang. view, $\times 67$. 2. Fungal tubercle in median section, $\times 67$. 3. Antheridium with sperm cells, $\times 262$. 4. Cap cell of antheridium, surface view, $\times 262$. 5. Antheridium in median long. sect. $\times 262$. 6a and b. Details of fungus in tubercle cells, $\times 262$.

lower part of the shaft. The endophyte is present both in the surface cells and also in the interior cells of the tubercle, being more abundant in the latter. It does not penetrate between the cells. Thomas states that fine hyphae 'may be traced in the cells of the lower half of the prothallium'.

In the specimen here described, however, the fungus scarcely extends beyond the tubercle itself, although very fine hyphae are sparsely present in some of the cells of the crown. In the tubercle there are two kinds of fungal hyphae, viz. those that are fine and thread-like, and others of a much coarser kind. The former, in a few of the cells at the very base of the shaft (Figs. 1 and 6 *b*), take the form of a definite coil. In the tubercle itself only a few short fine threads are discernible, but instead there is abundant granular cell-contents which may possibly represent disintegrated coils. Thomas mentions that in his specimens the tubercle cells appear partly exhausted. The coarser hyphae are few in number, and show no tendency to clump together. Sampson notes that both kinds were present in the upper part of the old gametophyte examined by him, and he interprets the coarser kind as representing a second fungal species other than the true endophyte. In the gametophyte described in the present paper, the coarse hyphae are in some instances dilated at their ends (Fig. 6 *a*). A few large thin-walled spore-like bodies are present in the tubercle, which may be taken as belonging to this second, coarser fungus.

STRUCTURE OF THE MATURE ANTHERIDIUM.

The antheridia are completely sunk below the surface of the crown. They are present in considerable numbers, but most are empty. So closely are they packed that usually only a single layer of crown tissue separates each from its neighbours. The antheridium marked A in Fig. 1 is more or less mature: it is shown in somewhat oblique view in Fig. 3, from which it is clear that it contained a large number of sperm cells. There is a one-layered outer cover to the antheridium, in which a definite cap cell is present (Fig. 4). An antheridium whose cap-cell has broken down is shown in median longitudinal view in Fig. 5 (marked B in Fig. 1).

COMPARISON WITH THE *CERNUUM* SECTION OF THE GENUS *LYCOPodium*.

The special interest of Thomas's account of the gametophyte and embryo of *Phylloglossum* is that it indicates clearly that this peculiar plant is closely related to the *Cernua* section of the genus *Lycopodium*. The gametophyte and sex organs are very similar in the two cases, and Thomas's brief reference to the young embryo of *Phylloglossum* (6, p. 288) is sufficient to show that it corresponds closely to that of *L. cernuum*, a 'protocorm' being formed in both cases before the stem apex is differentiated.

Thomas states that the gametophyte of *Phylloglossum* varies much in external form, the shaft especially being very variable in its length. Some of the gametophytes found by him were short and thickset, others being up to 6 mm. long. As referred to above, the writer has described the

same kind of variation in the gametophyte of *L. cernuum* (1, Figs. 17-21). In both *L. laterale* and *L. ramulosum* the departure from the typical *cernuum* form is sometimes still more noteworthy (3, Figs. 46-59). The *Phylloglossum* gametophyte is peculiar in the invariable absence of leafy lobes on the crown, but it is to be noted that these lobes are sometimes absent from the elongated gametophytes of *L. ramulosum* also (e.g. 3, Fig. 70). In view of the marked plasticity shown by the *cernuum*-type of gametophyte, that of *Phylloglossum* may be regarded as conforming essentially to this type. The mature antheridium of *Phylloglossum* and its manner of opening, as figured in the present paper, corresponds closely with Treub's original description of that of *L. cernuum* (7).

In three New Zealand species of *Lycopodium* belonging to the *Cernua* section, viz., *L. cernuum*, *L. laterale*, and *L. ramulosum*, the endophytic fungus invariably forms coils in the surface cells of the tubercle, but in surface cells only. It spreads into the immediately adjoining tissues, but in these it is altogether intercellular (3, p. 227 and Figs. 39-44, 46-7 66-71). In all three species it may spread somewhat from the basal tubercle up the shaft, or there may be distinct swollen areas in the shaft inhabited by the fungus. Wherever it occurs in these species it is always intracellular in the surface cells only, and intercellular in the underlying tissues. Moreover, in *L. cernuum*, and to a less extent in the other two species mentioned, there is a well-marked differentiation of structure in the fungus-bearing tissues. The hypodermal layer, in which the fungus is intercellular, especially in *L. cernuum*, forms a very distinct 'palisade' (see 3, Figs. 39-44, 68), comparable to that which occurs in the gametophyte of those species of *Lycopodium* which belong to the *clavatum* and *complanatum* types, although to a much less degree.

In *Phylloglossum* the infection of the basal part of the gametophyte by the fungus is of a different nature. All of the tubercle cells may contain the hyphae in their cavities, and there is no sign of hyphae located between the cells. Nor is there any approach to the 'palisade' structure. Here this less specialized nature of the fungal infection may be taken to indicate that in its gametophyte generation *Phylloglossum* has departed to a less extent from the ancestral autotrophic form than have some of its nearest relatives in the *Cernua* section of *Lycopodium*. In *L. salakense*, however, according to Treub (8) there is no fungus at all in the gametophyte.

With respect to the young sporophyte of *Phylloglossum*, an important point to note in Thomas's description is the statement that the stem apex originates 'inside the lower part of the embryo' (6, p. 288), i.e. inside the protocorm, whereas of course in the young sporelings of *L. cernuum* and related species it arises in a more normal position on the upper surface of the protocorm among the bases of the protophylls (e.g. 1, Plates XVII and XVIII). Sampson has suggested (4, p. 330) on anatomical grounds that

the annual tuber of *Phylloglossum* must be regarded as a modified branch, and so is not to be homologized with the true protocorm. *Phylloglossum* is a geophyte, dying down to its tuber immediately after shedding its spores at the onset of the dry season, and emerging again from the resting stage at the beginning of the winter. The annual tuber with its 'internal', curiously protected, apex of growth, can be regarded, as Sampson points out, as a specialization to the geophytic habit. In accordance with this point of view *Phylloglossum* is not 'a permanently embryonic form of Lycopod'. The internal origin of the stem apex in the *Phylloglossum* sporeling, referred to by Thomas, can conceivably be regarded as belonging to the same phenomenon of specialization, which has become so impressed upon the constitution of the plant as to make its appearance even before branching is initiated. This point in the interpretation can only be suggested tentatively until Thomas's statement, quoted above, is elucidated by figures.

The true protocorm itself seems to be a specialization of a different kind. The writer has concluded (2, p. 189) from a study of the young sporelings of the three New Zealand protocormous species mentioned above, that Bower's estimate of the protocorm is the most reasonable one, viz. that its significance is physiological rather than phylogenetic. But even so, judging from its occurrence throughout the *Cernua* section of *Lycopodium* and also in *Phylloglossum*, it is a more archaic structure than is the annual tuber of this latter genus. In *Phylloglossum* it would appear that the resting tuber habit has been superimposed upon the older protocorm habit. Sampson has concluded (5, p. 607), from a study of the young prothallial sporeling examined by him, that the tuber is an organ of a different nature to the protocorm, since they are apparently both present in his sporeling.

SUMMARY.

1. A single mature gametophyte of *Phylloglossum Drummondii* is figured and described.
2. The mature antheridium is also figured and described.
3. A comparison is instituted between *Phylloglossum* and the *Cernua* section of the genus *Lycopodium*.

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Transpiration and Pressure Deficit. II.¹

BY

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With seven Figures in the Text.

PRELIMINARY results on the relation between transpiration rate and pressure deficit have already been published (4). The results obtained showed the necessity for systematic experiments on gradually increasing and sustained constant deficits. Such experiments are recorded in the present communication in Series III-V, and the results discussed in the light of the additional experiments of Series VI. The apparatus used has already been described (*loc. cit.*).

Series III. Experiments on the Effects of Gradually Increasing Deficits.

Preliminary experiments on gradually increasing deficits were performed with the pressure cylinder using the self-recording potometer (3) to measure the absorption rate, the branch being specially prepared by the dissection method (4, p. 219). The branch was ringed at the level of the wax seal, the pith excised at the same level and the cavity filled with wax, thus preventing errors due to leakage of air under pressure through the intercellular spaces of the branch into the potometer. A constant rate was first obtained for half an hour at atmospheric pressure, and the pressure was then stepped up at two-minute intervals, one reading being taken at each pressure. The different experiments are, of course, all on different branches freshly set up.

The results are given in Table I.

In further experiments on *Acer*, as an additional precaution, the branches were ringed and vaselined below the seal, as well as having the pith excised at the seal level as in the experiments of Table I. With these precautions it was shown that there was no escape of air into the potometer at any pressures up to 140 lb./in. The pressure was stepped up at two-minute intervals as above. The results are given in Table II.

¹ From the Botanical Department, Queen Mary College.

TABLE I.

Showing Absorption¹ Rates by Acer at Gradually Increasing Pressure Deficits, the Pressure being Stepped up at Two-minute Intervals.

Experiment 1.			Experiment 2.			Experiment 3.		
Press. def.	Rate obs.	Rate %.	Press. def.	Rate obs.	Rate %.	Press. def.	Rate obs.	Rate %.
0	2.4	100	0	2.6	100	0	4.5	100
5	1.6	66.6	5	2.22	84.6	5	3.4	75.5
10	1.2	50	10	1.33	51.1	10	2.65	58.9
15	1.1	45.8	15	1.31	50.4	15	2.5	55.5
25	0.86	36.5	20	1.16	44.6	25	2.3	51.0
35	0.85	35.5	30	1.10	42.3	35	2.2	48.8
45	0.83	34.6	40	0.94	36.2	45	1.9	42.2
55	0.80	33.4	50	0.85	32.7	55	1.8	40.0
65	0.77	32.1	60	0.63	24.2	70	1.75	38.8
75	0.66	26.1	70	0.57	21.9	100	1.5	33.3
85	0.50	20.8	80	0.49	18.9			
			90	0.44	16.9			
			100	0.42	16.0			

The results show a regular decrease in absorption rate with increasing deficit. It is also evident that the rate at any one deficit if maintained would not be constant but would increase. This is seen in Expts. 7 and 10 in which it will be noted that in cases where absorption altogether ceased under the pressure, and expulsion of water took place, absorption recommenced after a period of only a few minutes although the pressure was maintained constant. The rate reached after a reversal in this way might even shortly become greater than the rate shown at the preceding (lower) pressure (Expt. 7). With one of the plants used reversal resulted on every increase of the pressure above 10 lb./in., but the readings obtained two minutes after the increase in each case formed a normal descending curve (Expt. 10). It will be noticed that the individual plants appear to vary considerably in respect of the percentage of the original absorption rate which is induced by a given increase in pressure deficit.

These broad results are shown also by the experiments made by the eosin method (4, p. 219), described in the following series (Series IV, V).

The fact that in these preliminary experiments reversal appears to be induced by different pressures with different individuals of the same species is probably due to the different specimens being unequally turgid at the outset. These inequalities were guarded against more particularly in the later experiments (Series IV, V). It will be realized that the more perfectly turgid the leaf cells at the beginning of the experiment the more easily will reversal be induced by a sudden increase in pressure, as the applied pressure is being added to a greater pre-existing turgour pressure

¹ See (4), pp. 214, 218.

TABLE II.

Showing Absorption¹ Rates by Acer at Gradually Increasing Pressure Deficits, the Pressure being Stepped up at Two-minute Intervals. Branches with Excised Pith and Ringed. Automatic Potometer Readings.

Experiment 4.			Experiment 5.			Experiment 6.		
Press. def.	Rate obs.	Rate %.	Press. def.	Rate obs.	Rate %.	Press. def.	Rate obs.	Rate %.
0	1.9	100	0	4.2	100	0	2.7	100
5	1.5	79	10	2.0	47.6	10	1.33	49.2
15	1.2	63	20	1.64	39.0	20	0.88	32.6
25	0.83	44	30	1.60	38.0	30	0.83	30.8
75	0.78	41	40	1.5	35.7	40	0.81	30.0
100	0.66	35	50	1.42	33.8	50	0.79	29.3
			60	1.17	27.9	60	0.77	28.5
			70	1.11	26.4	75	0.675	25.0
			80	1.0	23.8	100	reversed. ²	
			110	0.625	14.9			
			125	0.595	14.2			
Experiment 7.			Experiment 8.			Experiment 9.		
Press. def.	Rate obs.	Rate %.	Press. def.	Rate obs.	Rate %.	Press. def.	Rate obs.	Rate %.
0	3.0	100	0	2.3	100	0	2.3	100
10	1.11	37	10	1.33	57.8	10	0.83	36.1
20	1.10	36.6	20	1.20	52.1	20	0.74	32.2
30	1.09	36.1	30	1.05	45.6	30	0.57	24.8
40	0.92	30.7	40	0.92	40.0	40	0.45	19.75
50	0.69	23.0	50	0.8	34.8	50	0.402	17.5
60	0.62	20.7	60	0.78	33.9	60	0.398	17.3
75	reversed. ²		70	0.74	32.2			
75	0.83	27.8	130	0.29	12.6			
100	reversed. ³							
100	0.0	0.0						
100	0.8	26.7						
0	16.6	553						
0	10.5	350						
0	2.86	98.6						
Experiment 10.								
Press. def.	Rate obs.	Rate %.						
0	0.94	100						
10	reversed. ²							
10	0.33	35.2						
25	reversed. ²							
25	0.185	19.7						
50	reversed. ²							
50	0.16	16.9						

¹ See (4), pp. 214, 218.

² When reversal took place the pressure was only stepped up again after an interval of four minutes instead of two minutes. Thus all the readings given are at two-minute intervals, the number of readings at each pressure giving the time for which the pressure was maintained.

³ Six minutes at this pressure.

already making for back-flow. If transpiration were not very active and the leaf cells fully turgid, even a very small applied pressure might cause reversal *at first*, though there would rapidly follow an adjustment of leaf cell volume and therefore of suction pressure to the increased pressure so that upward flow would be re-established. This will be dealt with more fully later.

SERIES IV.

The Eosin Method.

The eosin method (see 4, p. 219, Method iii) of measuring the rate of absorption consisted in preparing the branch and fitting it into the pressure cylinder exactly as for the potometer method (4, p. 224), except that the branch was not dissected or ringed (4, p. 219). Time was allowed, as before (4, p. 224), for the branch to come into equilibrium with the conditions in the cylinder and acquire a constant absorption rate, and the base of the branch was then dipped into one per cent. eosin solution for one minute. At the end of one minute exactly, the last few cm. (according to the rate expected) were cut off under water with a special pair of cutting shears giving a clean cut. The cut piece was rapidly washed free of eosin, vaseline was quickly applied to the lower end to prevent further conduction, and the piece was rapidly sectioned back from the upper end with the same shears until the red colour of the eosin was first seen in the tracts exposed on the cut surface. The length of the remaining piece was then measured and taken as indicating the rate of conduction in cm./min. and therefore as proportional to the rate of absorption. After a little experience the height of the eosin column could be predicted so nearly before cutting that the process of sectioning back to find the exact position of the top of it occupied only from two to six seconds. It was shown independently that the further travel of the eosin in the detached piece when the end was vaselined, and the rest of the branch was removed, was always only of the order of 1–2 mm. in ten minutes, so the error introduced by the time required for the sectioning was negligible.

Sources of Error in the Eosin Method and Control Experiments.

All the possible sources of error in the eosin method of estimating the absorption rate appear to fall under the following headings :

1. Errors as in the preceding experiments (Series II) (4) due to inconstancy of the absorption rate, when no pressure is applied through (a) the allowance of insufficient time for a constant rate to be attained after setting up the branch, (b) inadequate illumination, or (c) inadequate drying re-agent in the cylinder.

2. Errors due to the taking up of water from the tracts by tissues

other than those of the actively transpiring leaves, for example, the phloem and non-turgid cells of the cortex and pith of the stems and petioles.

3. Errors due to the different lengths of the branch present at different times in the experiment arising from the cutting off of successive lengths for the taking of readings, the differences in length resulting in different resistances to conduction.

4. Errors which may be introduced through differences in the diameters of the vessels concerned at different distances from the base of the branch. If the actively conducting vessels be of smaller diameter at one point than another, a similar rate of absorption by volume will correspond to a greater linear rate of conduction.

5. Errors due to spontaneous diffusion of the eosin solution up the tracts independently of the conduction current.

6. Errors due to the surface tension of the eosin solution. If the surface tension of the eosin solution, for example, be appreciably less than that of water it might be expected that the column of solution would be drawn up the tracts by the water column which would recede above it through the capillarity effect at the interface between the solution and the water.

7. Errors due to movement of the eosin column in the cut piece of branch after an experiment before measurement.

8. Errors due to irregularity in the rate of entry of eosin into the branch at constant pressure.

These sources of error can be eliminated or allowed for as follows :

1. The errors coming under this heading have already been discussed (4, pp. 225-9), and can be eliminated by adequate light and drying material and the allowance of sufficient time before beginning to take readings as already described (*loc. cit.*, p. 224). Their elimination is proved by control experiments (see *loc. cit.*, Tables I, II, III, pp. 226, 227, 229, and below, Tables IV, V, VI).

2. Errors due to this cause are also eliminated by allowance of sufficient time before experiments for unsaturated cells to become fully turgid.

3. On the usual assumption that the stem does not aid conduction, these errors could only arise from the different resistances to flow offered by different lengths of stem. They are dealt with by control experiments (see Tables IV-VI, below), in which the rates of absorption are followed up on a branch at all lengths as it becomes shorter and shorter during the experiment, all the readings being taken with atmospheric pressure in the cylinder.

4. The mean diameters of the vessels could not alone account for differences in the rate of conduction during a given fixed rate of absorption since the *number* of vessels concerned in any one cross-section would also

be concerned. The relevant factor would be the total area of lumen actively concerned with conduction. This cannot be experimentally compared at different heights by measuring the areas stained at different heights in a branch which has been allowed to take up stain owing to the considerable lateral diffusion of the stain that takes place at the lower levels while the stain is reaching the upper. To dispose of this criticism, therefore, reliance must be made upon the control experiments (Tables IV-VI), which indicate that as long as no pressure is applied in the cylinder the rate of conduction remains the same, though necessarily estimated at higher and higher points in the branch.

5. The spontaneous diffusion of eosin solution in the tracts was investigated by excising individual internodes of the branches concerned, and leaving them standing in water in an upright position with only the tips just above the water surface for half an hour. This ensured complete saturation of the pith and cortical tissues, &c., and filling of the tracts as in the experiments. The upper ends were then thoroughly vaselined, and the internodes taken out of the water and dipped by their lower ends into one per cent. eosin solution for one minute. They were then taken out and rapidly washed and sectioned back from the upper end as when obtaining the experimental readings. These experiments showed that, provided that actual conduction was effectively prevented by vaseline, and provided that the internode used was fully saturated with water to start with, there was never any detectable amount of spontaneous diffusion of the eosin. If the internode were rapidly washed when taken out from the eosin and cut away with the shears from the lower end which had been dipped in the stain, no stain could ever be detected as having entered the tracts even though only half a millimetre was cut away.

6. The surface tension of the eosin solution used was determined by the method of Ferguson and Kennedy (1). The determinations showed that the surface tension of the solution was very slightly *above* that of the water used for the irrigation of the branches in the experiments, the value for the water being 72 dynes/cm. and that for the solution 73.6. Such force as existed at the surface of the eosin column in the tracts would therefore tend, not to increase the rate of penetration of the solution, but to decrease it. In any case this very small retarding force exerted by the eosin-water interface would remain constant throughout an experiment, unless during penetration the column reached tracts of progressively greater or progressively smaller mean diameters, so that the retarding force would be respectively progressively diminished or increased. The mean diameters of the tracts taking up the stain were measured in the species used at different heights in the branches, and no such regular increase or reduction in the values was found. The diameters of 100 stained tracts from each of a number of successive levels ten cm. apart

were measured in each case with an eyepiece micrometer. The results for *Eupatorium* were typical of all those obtained, and are given in Table III.

TABLE III.

Distance from apex	= 10	20	30	40	50	60	cm.
Mean diameter of stained vessels	= 4.07	4.16	4.17	4.02	4.01	4.36	units

The already minute retarding force could therefore only vary at the most within ten per cent. Changes in the forces due to unequal surface tension effects of the eosin-water interface at different heights could not therefore appreciably modify the results.

Series IV. Control Experiments, Group A.

TABLE IV.

Control Experiments on Acer showing Relation of Absorption Rate to Length of Branch when Pressure in Cylinder = atm. throughout.

Experiment 1.			Experiment 2.			Experiment 3.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
50	10	100	55	12	100	45	7.8	100
45	10	100	45	12	100	35	7.5	96
40	10	100	40	10.2	85	25	7.8	100
35	10	100	35	10.2	85	15	7.8	100
30	10	100	25	9	75			
25	10	100	15	8.4	70			
			10	10.2	85			

Ditto using *Aesculus*.

Experiment 4.			Experiment 5.			Experiment 6.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
45	2.0	100	60	1.5	100	60	2.0	100
40	2.0	100	55	1.6	107	55	2.0	100
35	2.1	105	50	1.4	93	50	2.0	100
30	2.2	110	45	1.6	107	45	1.9	95
20	2.2	110	40	1.5	100	35	1.8	90
15	1.9	95	35	1.5	100	30	1.8	90
			30	1.5	100	25	1.8	90
			25	1.5	100	20	2.0	100
			20	1.2	80	15	1.8	90
			15	1.5	100			
			10	1.4	93			

TABLE IV (*continued*).

Experiment 7.			Experiment 8. (With many leaves.)		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
50	6.0	100	50	2.0	100
45	6.0	100	45	2.0	100
40	5.8	97	40	2.3	115
30	5.8	97	35	2.8	140
20	6.0	100	30	2.8	140
10	6.0	100	25	3.0	150
			20	—	—
			15	3.1	155
			10	3.3	165
			5	3.8	190

TABLE V.

Control Experiments as in Table IV on Eupatorium adenophorum.

Experiment 1.			Experiment 2.			Experiment 3.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
25	6.0	100	36	6.0	100	26	9.2	100
20	7.0	117	26	6.2	103	19	9.2	100
10	6.0	100	20	6.5	108	15	9.0	98
7	6.0	100	16	6.3	105	5	9.2	100
			11	6.0	100			
Experiment 4. ¹			Experiment 5.			Experiment 6.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
37	10	100	30	48	100	35	14	100
28	10	100	20	48	100	25	14	100
17	10	100	5	44	92	15	13.2	94
8.5	11.5	115						
Experiment 7.			Experiment 8.					
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.			
31	9.0	100	26	11.4	100			
25	9.4	104	18	12.8	112			
15	10.0	110	11	14.4	126			

7. Errors might be introduced through a further movement of the column of eosin solution in the detached piece of branch between the moment of detachment from the rest of the branch, and the time at which the length of the column is read after sectioning back. To avoid these errors the piece of branch cut off was coated quickly with vaseline at both ends, the lower end first, as soon as it was cut. To test whether any appreciable further movement of the column took place in these conditions

¹ Stem stripped for direct observation method, see p. 530, and also (4), p. 219.

TABLE VI.

Control Experiments on Privet (Ligustrum) showing Relation of Absorption Rate to Length of Branch when Pressure in Cylinder = atm. throughout.

(a) Readings at five-minute intervals.

Experiment 1.			Experiment 2.			Experiment 3.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
45	3.5	100	30	1.1	100	50	1.7	100
35	3.3	94	25	1.1	100	45	1.7	100
25	3.3	94	20	1.1	100	40	1.5	88
15	3.4	100	15	1.1	100	35	1.8	106
			10	1.1	100	30	1.6	94
			5	1.1	100	25	1.6	94
						20	1.6	94
						15	1.7	100
						10	1.7	100
						5	2.0	118

(b) Readings at longer intervals against time as indicated.

Experiment 4.				Experiment 5.			
Time in min.	Length (cm.).	Rate.	Rate %.	Time in min.	Length (cm.).	Rate.	Rate %.
0	80	5.8	100	0	50	3.2	100
10	72	5.8	100	30	45	3.2	100
20	63	5.7	98	60	40	3.2	100
30	56	5.8	100	90	35	3.2	100
42	49	5.9	102	120	30	3.2	100
60	42	5.8	100	150	25	3.2	100
70	36	5.7	98	180	20	3.2	100
80	30	5.7	98	210	15	3.2	100
95	24	5.5	95	240	10	3.0	96

Experiment 6.

Time in min.	Length (cm.).	Rate.	Rate %.
0	50	2.3	100
30	45	2.3	100
60	42	2.3	100
90	39	2.3	100
120	36	2.3	100
150	33	2.1	91
180	30	2.3	100
210	27	2.3	100
240	24	2.3	100
270	21	2.3	100
300	18	2.2	96
330	15	2.1	91

a number of successive pieces of equal length were cut from the same branch after being allowed to take up the stain for one minute in each case, and all were immediately vaselined. The sectioning back and determinations of the lengths of the eosin columns were then made in the different samples after varying intervals of time. Even if the samples were left for five minutes before measuring it was found that the length of the column never differed from that in the sample measured immediately by more than 1-3 per cent. As in all the experiments the measurements were made immediately after cutting the sample from the branch, it was therefore concluded that this possibility could not constitute a source of error of any importance.

8. There remains the possibility that the eosin column might have penetrated more or less suddenly to the distance observed within the first few seconds of immersion of the end of the branch in the dye, and then slackened off or stopped altogether. In this case the distance penetrated would not be proportional to the time allowed (nearly always one minute), and would not, therefore, be a measure of the rate of conduction or absorption. This sort of behaviour is observed if indian ink be used instead of eosin, and is due to the clogging of the tracts by the particles of ink. That this was not the case when eosin was used was abundantly shown by control experiments (which need not be given in detail) in which successive pieces were cut after varying intervals of time from the bases of branches absorbing at a constant rate. The distances to which the stain had penetrated were directly proportional to the times within \pm five per cent. in all cases. The point was also proved in some cases by estimating the rate of conduction by stripping off the bark at the base of the branch for a distance of about 20 cm., and observing the travel of the stain in the superficial tracts with the naked eye. It was evident from such experiments that the rate of flow was steady and uniform, this conclusion being amply borne out by plotting the readings of the length of the column of stain in the superficial tracts as measured with a ruler against time. The readings in some of the experiments on *Eupatorium* were taken in this way (as indicated in Table V), and also on *Aesculus* (as indicated in Table IX, Expt. 12). These all fall well into line with those taken by the usual method.

Incidentally, the enormous increases in the absorption and conduction rates as indicated by the penetration of eosin found to occur as a result of decreases in the pressure deficit (Table IX, Expt. 11; Table X, Expts. 9, 10, and 12; Table XV, Expts. 5 and 6; Table XVII, Expts. 1, 2, 3, 5, and 7; Table XIX, Expts. 7 and 9; and Table XX, Expt. 10) completely remove any misgivings as to the possibility of a falling off in the rate of penetration normally occurring as a result of clogging of the tracts by the stain, or from any other cause.

The results of the control experiments given in Tables IV–VI show, that provided the precautions mentioned be duly observed, the rate of absorption as indicated by the rate of conduction of eosin solution remains sufficiently constant when no pressure is applied in the cylinder independently of time and the length of the branch.

It is, however, important not only that the illumination and the drying agent be efficient and that the plant be fully saturated before the experiment, but also that only a relatively few leaves be left on the experimental branch. If too many leaves be used, not only is the humidity of the air in the cylinder inclined to rise, thus causing a decrease in the rates of transpiration and absorption, but the resistance of the stem may then come to have a greater effect upon the rate of conduction and, through being so much greater compared with that of the leaves, may cause minor differences in the rate of transpiration to escape notice. The rate of conduction will be decided by the force developed in transpiration or the 'transpiration pull' on the one hand and the *total* resistance to the flow of water on the other, the law connecting the quantities being similar to Ohm's Law in electricity (cf. 6, and 4, p. 217¹):—Conduction Rate = Transpiration Pull/Total Resistance. The total resistance, however, will be that offered by the leaves + that of the conducting tracts of the stem. In order then that the varying length of the stem used in the experiments may not appreciably alter the total resistance, the resistance of the stem, which varies, must be made as small as possible in comparison with that of the leaves, which is constant.² A large stem should therefore be employed, capable of supplying a large number of leaves, and the number of leaves should be reduced to such a point that the resistance of the stem to the passage of such small quantities of water as the remaining leaves would transpire would be infinitesimally small in comparison with that offered by the tissues of the leaves themselves. The results of ignoring these various precautions are brought out by the experiments recorded in Table VII.

The figures show that if the branch is imperfectly saturated at the beginning of the experiment there is a gradual falling off of the absorption rate, since early in the experiment water is required both to complete the saturation of the branch and also for transpiration, while later it is required for transpiration alone. (Table VII, Section *a*, Expts. 1–4.)

If the plant has too many leaves the resistance of the leaves is small in comparison with that of the stem. Consequently the reduction in the length of the stem during the experiment materially reduces the total resistance, and an increase in the rate of absorption results, as seen in Experiments 1, 2, and 3 of Table VII, Section *b*.

¹ Cf. also below, however, p. 561.

² Or at least is not altered artificially.

Series IV. Control Experiments, Group B.

TABLE VII.

*Special Experiments to Demonstrate the Effects of Sources of Error.*All on *Eupatorium adenophorum*.Section *a*. Experiments with imperfectly saturated branches, showing decrease in rate as saturation proceeds.

Experiment 1.			Experiment 2.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
30	13	100	21	24	100
18	10	77	16	15	62.5
12	6	46	11	13.2	35
6	4.8	37	7	10	41.6
3	4.0	31	4	6	25

Experiment 3.			Experiment 4.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
28	25	100	20	66	100
20	16	64	15	36	54.5
12	11	44	10	34	51.5
6	10	40	5	28	42.5

Section *b*. Experiments on plants with too many leaves, showing increase in rate with decrease in the resistance due to the stem.

Experiment 1.			Experiment 2.			Experiment 3.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
20	10	100	20	12	100	20	40	100
15	14	140	15	14	117	10	50	125
10	18	180	10	18	150	5	60	150
5	24	240	5	20	167			

Section *c*. Experiments with unsaturated branches bearing too many leaves.

Experiment 1.			Experiment 2.		
Length (cm.).	Rate.	Rate %.	Length (cm.).	Rate.	Rate %.
40	30	100	30	48	100
35	27	90	25	36	75
30	24	80	20	30	62
25	18	60	15	28	58
20	13	43	10	50	104
15	9.6	32			
8	12	40			
7	30	100			

TABLE VII (*continued*).Section *d*. Experiment on an unsaturated vaselined branch with no leaves.

Length (cm.).	Rate.	Rate %.
35	36	100
30	21	58
25	12	33
20	6	17
15	10	28
10	12	33
5	14	39

If the branch is unsaturated and also bears too many leaves the rate of absorption, as would be expected, first decreases and subsequently increases again. (Table VII, Section *c*.) This is evidently to be explained as suggested in the accompanying graph (Fig. 1) as the result of two tendencies: one for the rate to decrease with increasing degree of saturation of the tissues, this rate of decrease falling off with time; and one for the rate to increase in linear relation to time as the resistance of the stem is reduced by removal of lengths for measurements. The results of these experiments have been particularly instructive and helpful in tracing the causes of occasional discordant results in later experiments.

The results of one experiment on an unsaturated vaselined branch with no leaves are also given. (Table VII, Section *d*.) These results are typical of several similar experiments and are in accordance with the same conception. Here, however, the trend of the readings is the resultant of three factors instead of only two. In addition to the two mentioned above, as the whole of the unsaturated tissue present is now represented by that of the pith, phloem, and cortex of the stem itself, there will be a third tendency (towards a decrease in rate) as the unsaturated tissues (responsible for the uptake) are regularly reduced in quantity by the removal of samples of stem for the measurements. This will have the effect of multiplying the graph of the type shown in Fig. 1 by a further descending curve of decreasing slope representing a tendency to a reduction in absorption rate with time as a result of the decrease in the amount of remaining unsaturated tissue. (The rate of this reduction would fall off with time as the remaining tissue became saturated.) The result would therefore be expected to be a decrease in the absorption rate at first where the fall of the descending curve is sharpest, followed by a rise. The rise, however, would be less pronounced than in the previous case, since in the latter the leaves provided the greater part of the unsaturated tissues responsible for the uptake and those of the (stem now present alone) were relatively insignificant. That this is the case is shown by the results quoted in the table. (Table VII, Section *d*.)

Series IV. Actual Experiments.

In this series are given the results of pressure cylinder experiments in which the rates of absorption were measured at different pressure deficits as the pressure in the cylinder was gradually increased. The deficits, it

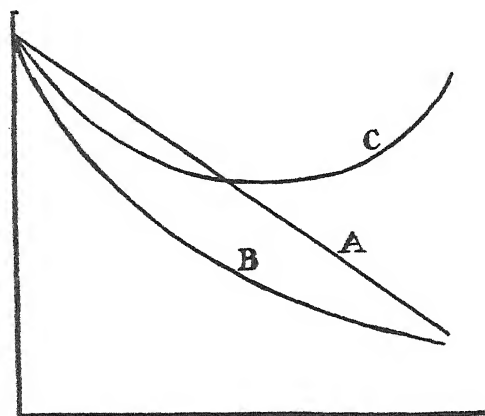


FIG. 1. Hypothetical curves to show expected variations in absorption rate with increasing saturation of tissues and decreasing resistance to flow. A = decreasing resistance. B = decreasing force due to unsaturated tissues. C = resulting rate of conduction = B/A . All quantities are plotted against time.

will be observed, are equal to the applied pressures as read on the gauge, since the gauge only measures the excess above atmospheric pressure. A constant rate at atmospheric pressure was obtained first. The pressure was then increased by convenient increments at intervals of two minutes (except in a few cases as otherwise stated in the tables), and the absorption rates under the different pressures were measured by the eosin method immediately after the pressure was raised to the next value in each case. Each change of pressure took approximately half a minute.

The materials used were branches of *Acer pseudoplatanus*, *Aesculus hippocastanum*, *Eupatorium adenophorum*, and *Ligustrum* sp.

In the cases of *Aesculus* and *Acer* the experimental material was gathered throughout at a height of 12–15 ft. from the ground, care being taken to select material which was as uniform as possible. The lengths of the internodes and of individual year's growths at the base of the branches selected, as well as diameter, appeared to be the most reliable criteria indicative of physiological uniformity. In the case of the Privet, (*Ligustrum*) all material was gathered at a height of 4 ft. from the ground. In this and in *Eupatorium* uniform material was secured by the utilization of the side axes resulting from the removal of the top of a main branch. The *Eupatorium* was grown from cuttings from plants supplied by the Chelsea Physic Gardens; the privet bushes (supplied by Messrs. Carter,

Ltd.) were treated as indicated above sixteen months before being taken into use.

The results are recorded in the following tables (Tables VIII–XIV).

TABLE VIII.

Results of Experiments on Sycamore (Acer pseudoplatanus) showing Rates of Absorption at Different Pressures relative to the Rate at Atmospheric Pressure, the Readings being taken at Two-minute Intervals immediately after Raising the Pressure.

Experiment 1. ¹				Experiment 5. ³			
Press. in lb./in.	Length (cm.).	Rate.	Rate %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	100	20	100	0	80	2.6	100
20	90	10	50	20	70	2.1	81
40	80	5.5	27.5	40	65	1.7	65
60	70	2.4	12	60	55	1.0	39
80	60	1.2	6	80	50	0.7	27
100	55	0.8	4	100	45	0.6	23
120	50	0.5	2.5	120	40	0.5	19
140	45	0.4	2	140	35	0.4	15
160	40	0.35	1.7	170	30	0.1	4
180	35	0.2	1.0	190	25	0.0	0
200	30	0.1	0.5				
220	25	0.0	0.0				

Experiment 2.				Experiment 6.			
Press. in lb./in.	Length (cm.).	Rate.	Rate %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	50	2.6	100	0	40	3.9	100
20	45	1.4	54	20	35	1.4	36
40	40	1.2	46	40	30	0.6	15
60	35	0.5	19	60	25	0.0	0
80	30	0.4	15				
120	23	0.2	7.7				
150	20	0.0	0.0				

Experiment 3.				Experiment 7.			
Press. in lb./in.	Length (cm.).	Rate.	Rate %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	50	2.8	100	0	65	2.7	100
20	45	1.4	50	30	55	1.5	55
40	40	0.9	32	50	50	1.2	45
60	35	0.6	21	80	45	0.7	26
80	30	0.4	14	100	40	0.5	18.5
100	25	0.3	11	120	35	0.4	15
120	20	0.2	7	140	30	0.2	7.5
140	15	0.0	0	170	25	0.0	0

Experiment 4. ²				Experiment 8.			
Press. in lb./in.	Length (cm.).	Rate.	Rate %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	55	5.3	100	0	50	2.9	100
20	50	3.8	72	20	45	1.9	65
40	45	2.5	47	40	40	1.2	41
60	40	1.5	28	60	35	0.7	24
80	35	1.2	23	80	30	0.5	17
100	30	0.6	11	100	25	0.1	3.5
				120	20	0.0	0

¹ Osmotic value = 12 atm. (= 180 lb./in.).

² The last readings of Expt. 4 were invalidated through leakage of the seal and have, therefore, not been recorded.

³ Osmotic value = 11 atm. (= 165 lb./in.).

TABLE VIII (*continued*).

Experiment 9.				Experiment 11.			
Press. in lb./in.	Length (cm.).	Rate.	Rate %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	70	3.7	100	0	50	3.6	100
20	60	2.2	60	20	40	2.3	64
40	55	1.1	30	40	35	1.3	36
60	50	0.6	16	60	30	0.4	11
80	45	0.4	11	80	25	0.3	8
100	40	0.3	8	100	20	0.1	3
120	35	0.2	5.5	120	15	0.0	0
160	25	0.1	2.7				
180	20	0.0	0				

Experiment 10.				Experiment 12.			
Press. in lb./in.	Length (cm.).	Rate.	Rate %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	60	4.3	100	0	55	5.1	100
20	50	3.6	84	20	50	4.1	80
40	45	1.8	42	40	45	1.6	31
60	40	1.4	33	60	40	1.3	25.5
80	35	0.6	14	80	35	0.9	18
100	30	0.5	12	100	30	0.6	12
120	25	0.0	0	110	25	0.5	10
				120	20	0.0	0

TABLE IX.

Experiments on Aesculus showing Absorption Rates at Different Pressures relative to the Rate at Atmospheric Pressure, the Readings being taken at Two-minute Intervals immediately after Raising the Pressure.

Experiment 1.				Experiment 3.			
Press. in lb./in.	Length (cm.).	Rate.	Rate %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	35	1.7	100	0	40	1.1	100
15	30	0.9	53	20	35	0.7	64
30	25	0.3	17.5	40	30	0.4	36
45	22.5	0.2	12	60	25	0.2	18
50	20	0.1	6	80	20	0.1	10
60	17.5	0.05	3	100	15	0.0	0
80	15	0.02 ca	1				
100	12.5	0.0	0				

Experiment 2.				Experiment 4.			
Press. in lb./in.	Length (cm.).	Rate.	Rate %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	60	1.3	100	0	60	7.0	100
20	55	0.7	54	20	55	6.5	93
40	50	0.5	38	40	50	6.0	86
60	45	0.4	32	60	45	5.0	71
80	40	0.3	23	80	40	4.0	57
100	35	0.2	15	100	35	1.0	14
120	30	0.1	8	120	30	0.0	0
140	25	0.0	0				

TABLE IX (*continued*).

Experiment 5.				Experiment 9.			
Press. in lb./in.	Length (cm.).	Rate.	Rate. %.	Press. in lb./in.	Length (cm.).	Rate.	Rate %.
0	50	3.0	100	0	55	21	100
20	45	0.7	23	20	50	9	43
40	40	0.5	17	40	45	7	33
60	35	0.3	10	60	40	6	29
80	30	0.3	10	80	35	5	24
100	25	0.3	10	100	30	3	14
120	20	0.2	7	120	25	1	5
140	17.5	0.1	3	140	20	0	0
160	15	0.0	0				
Experiment 6.				Experiment 10.			
0	35	11	100	0	40	13	100
20	30	5	45	20	35	8	62
40	27	2	18	40	30	5	39
60	23	1.5	14	60	25	4	31
80	19	1	9	80	22	3	23
100	14	0.5	4	100	18	2	15
120	10	0.0	0	120	14	1	8
				140	10	0	0
Experiment 7. ¹				Experiment 11. ²			
0	40	19	100	0	75	12	100
20	35	7	37	20	70	10	83
40	30	6	32	40	65	8	67
60	25	3	16	60	60	7	58
80	20	2	11	80	55	5	42
100	15	1	5	100	50	3	25
110	10	0	0	120	45	2	17
				140	40	1	8.5
				160	35	0.5	4
				180	30	0.0	0
				200	25	0.0	0
Experiment 8.				Experiment 12. ³			
0	60	18	100	0	60	11.0	100
20	55	8	44	20	50	7.5	68
40	50	5	28	40	45	4.5	41
60	45	3	17	60	40	1.0	9.1
80	40	2	11				
120	35	1	5.5				
150	30	0.5	2				
180	25	0.0	0	120	30	0.0	0.0

¹ Initial Osmotic value = 7 atm. (= 105 lb./in.).

² Initial Osmotic value = 11 atm. (= 165 lb./in.).

³ Readings taken by measuring length of eosin column in superficial tracts of stripped stem with a ruler without sectioning (see p. 530).

TABLE X.

Experiments on Eupatorium adenophorum showing Absorption Rates at Different Pressures relative to the Rates under Atmospheric Pressure, the Increases in Pressure being made at Two-minute Intervals and Readings taken immediately after Raising the Pressure, except where otherwise stated.

Experiment 1.			Experiment 6. ¹		
Press. in lb./in.	Rate.	Rate %.	Press. in lb./in.	Rate.	Rate %.
0	6.0	100	0	6.0	100
100	1.2	20	75	3.6	60
200	0.3	5	150	2.5	42
250	0.1	1.7			
Experiment 2.			Experiment 7. ¹		
0	7.4	100	0	5	100
50	4.6	62	45	2.7	54
100	1.0	13.5	250	2.3	45
150	0.6	8.1			
200	0.4	5.4			
250	0.2	2.7			
Experiment 3.			Experiment 8.		
0	8.5	100	0	5.6	100
50	3.0	35	30	3.6	64
100	2.1	25	60	1.9	34
150	0.6	7	100	1.1	20
200	0.3	3.5	200	0.6	11
300	0.0	0.0	300	0.0	0
Experiment 4.			Experiment 9.		
0	2.2	100	0	6.8	100
20	1.1	50	15	4.7	69
60	0.8	36	30	3.2	47
120	0.6	27	45	1.9	28
180	0.05	2.2	60	1.6	24
240	0.025	1.1	90	1.4	21
300	0.0	0.0	150	1.2	18
			200	0.3	4.4
			300	0.0	0
Experiment 5.			0	10	147
0	3.4	100			
40	1.6	47			
80	1.0	29			
120	0.9	26.5			
150	0.3	8.8			
180	0.2	5.9			
250	0.1	2.9			
300	0.0	0.0			
Experiment 10.					
240	0.15	4.3	0	2.3	100
100	>3.0	>88	150	0.8	35
			0	5.0	217
Experiment 11.					
			0	7.4	100
			220	0.0	0

¹ In these experiments readings were taken at fifteen-minute intervals.

TABLE X (*continued*).

Experiment 12.			Experiment 13.		
Press. in lb./in.	Rate.	Rate %.	Press. in lb./in.	Rate.	Rate %.
0	3.5	100	0	16	100
20	2.0	57	0	16	100
40	1.5	43	20	10	62.5
60	1.2	34	40	7	44
80	1.0	29	60	4	25
90	0.7	20	80	3.5	22
15 minutes interval.			100	3	19
90	1.0	29	120	2	12.5
Pressure then raised to 135 lb./in. and allowed to fall slowly to 100 lb./in. during an interval of 1 hour.			140	1	6
100	2.2	63	160	0	0
0	7.0	200	180	0	0
Cut end then left out of water for 1 minute.			220	0	0
0	5.3	151	240	0	0
Tracts shown to contain air but con- duction at 151 % of original rate all the same.			260	0	0
			10 minutes interval, pressure slowly falling to 180 lb./in.		
			180	3	19
			Further 10 minutes interval, pressure slowly falling to 150 lb./in.		
			150	18	112

TABLE XI.

Experiments on Privet (Ligustrum) showing Rates of Absorption at Different Pressures relative to the Rates at Atmospheric Pressure, the Readings being taken at Two-minute Intervals.

Experiment 1.			Experiment 2.			Experiment 3.		
Press. in lb./in.	Rate.	Rate %.	Press. in lb./in.	Rate.	Rate %.	Press. in lb./in.	Rate.	Rate %.
0	1.6	100	0	4.0	100	0	3.5	100
20	0.6	37	20	1.6	40	20	2.1	60
40	0.1	6	40	1.0	25	40	0.7	20
50	0.0	0	60	0.0	0	55	0.0	0
						Left at 100 lb./in. for half an hour.		
						100	0.5	14

TABLE XII.

Experiments on Privet as in Previous Table, but Readings being taken at Three-minute Intervals.

Experiment 1.			Experiment 2.		
Press. in lb./in.	Rate.	Rate %.	Press. in lb./in.	Rate.	Rate %.
0	10	100	0	4.0	100
20	2.5	25	20	3.7	92
40	2.0	20	40	3.5	87
60	1.0	10	60	1.5	37
80	0.0	0	80	0.0	0

TABLE XIII.

Experiments on Privet as in Previous Table, but Readings being taken at Five-minute Intervals.

Experiment 1.			Experiment 2.			Experiment 3.		
Press. in lb./in.	Rate.	Rate %.	Press. in lb./in.	Rate.	Rate %.	Press. in lb./in.	Rate.	Rate %.
0	2.0	100	0	1.8	100	0	2.7	100
25	1.0	50	20	0.8	44	20	1.8	67
40	0.8	40	40	0.6	33	40	1.0	37
60	0.4	20	70	0.4	22	60	0.3	11
80	0.3	15	100	0.2	11	80	0.2	7.4
100	0.1	5	150	0.0	0	100	0.1	3.7
130	0.0	0				130	0.05	2
						140	0.0	0

TABLE XIV.

Collected Mean Results from Tables VIII-XIII, showing the Relation between Pressure Deficit and Absorption Rate.

The absorption rates are given throughout as percentages of the rates at atmospheric pressure and are calculated only to the nearest whole number, except where the numbers of experimental results averaged, and the agreement between them justifies, the recording of more significant figures.

Press. in lb./in.	0	20	40	60	80	100	120	140	160	180	200	240	250	300
<i>Acer</i>														
(2 min. intervals) ¹	100	66	40	23	15	10	4.6	2.1	1.1	0.3	0.05	0	0	0%
<i>Aesculus</i>														
(2 min. intervals) ²	100	55	39	30	22	11	5.0	1.5	0.5	0.0	0.0	0.0	0.0	0%
<i>Eupatorium</i>														
(2 min. intervals) ³	100	69	51	37	29	22	19	14	8	7	5	3	2	0%

¹ Means of Expts. 1, 2, 3, 4, 5, 8, 9, 10, 11, 12. (Table VIII.)

² " " 2, 3, 4, 5, 6, 7, 8, 9, 10, 11. (Table IX.)

³ " " 1, 2, 3, 4, 5, 8, 9, 13. (Table X.)

TABLE XIV (*continued*).

Press. in lb./in.	0	20	40	60	80	100	120	140	160	180	200	240	250	300
Eupatorium (15 min. intervals) ¹	100	83	67	60	55	52	49	46	43	40	37			
Privet (2 min. intervals) ²	100	46	17	0	0									
Privet (3 min. intervals) ³	100	56	53	23	0									
Privet (5 min. intervals) ⁴	100	57	37	19	13	7	3	0						

These results are plotted graphically in Fig. 2.

Discussion of Results of Series IV.

The leading results of the experiments of this series may be summarized as follows.

Increase of the pressure to which the leaves are exposed in the cylinder, and therefore of the pressure deficit (4, pp. 215, 217, 218), in all cases leads to a decrease of the rate of absorption of water from a supply at atmospheric pressure.

As the pressure deficit is increased the rate of absorption is regularly reduced, until at a certain pressure, uptake of water stops. As this point is passed further increase of the pressure in the cylinder causes water to be forced out of the cut end of the stem, the direction of flow being reversed.

The pressure at which reversal takes place under these conditions is for Eupatorium in the neighbourhood of 300 lb./in. (20 atm.), but there is considerable variation between the values for different samples of the material. In the case of Aesculus the reversal takes place at pressures of 100–180 lb./in. (approximately 7–12 atm.), and with Acer at pressures of 120–220 lb./in. (approximately 8–15 atm.). In the case of the privet (Ligustrum) three sets of experiments were performed in which the readings were taken and the increments of pressure applied after three different lengths of interval. The results show that the longer the intervals between the increases (i.e. the more slowly the pressure is raised) the greater is the pressure required to cause reversal and the less steeply does the absorption-deficit curve fall (Table XIV, and Fig. 2). Whereas 130–150 lb./in. are required to cause reversal when the pressure is increased

¹ Means of Expts. 6, 7. (Table X.) (Approximate : intermediate values computed for comparison.)

² „ „ 1, 2, 3. (Table XI.) (Approximate : intermediate values computed for comparison.)

³ „ „ 1, 2. (Table XII.) (Approximate : intermediate values computed for comparison.)

⁴ „ „ 1, 2, 3. (Table XIII.) (Approximate : intermediate values computed for comparison.)

at five-minute intervals (Table XIII), if the pressure be raised at three-minute intervals the value necessary is reduced to 80 lb./in. (Table XII), and if raised at two-minute intervals the pressure required comes down to

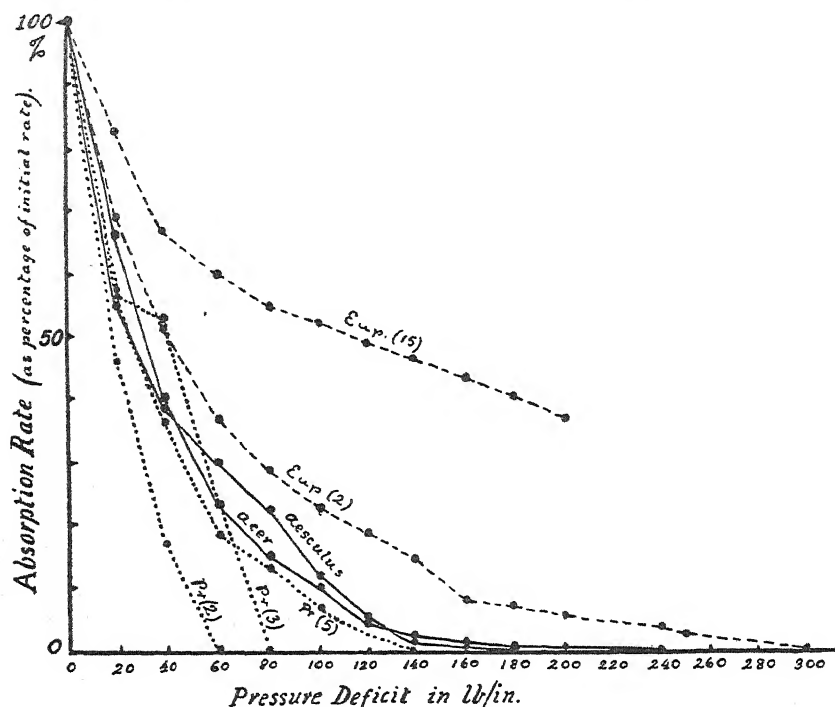


FIG. 2. Curves showing the relation between absorption rate and pressure deficit with increasing deficits plotted from the figures in Table XIV. The curves are for Acer, Aesculus, Eupatorium and Privet as indicated, the figures in brackets indicating the lengths of the intervals (in minutes) between successive increases of the pressure deficit.

50-60 lb./in. (Table XI). The same point is brought out by the curves for Eupatorium at two- and fifteen-minute intervals (Fig. 2; Table XIV). This means that some adjustment to the higher pressure is made by the plant. A pressure which causes reversal if applied suddenly will not do so if applied slowly, so that there is time for this adjustment to be made. This point will be more fully discussed below. It is particularly emphasized by Expt. 3, Table XI, in which it is seen that although more or less rapid raising of the pressure to 55 lb./in. may completely stop absorption, after half an hour under a pressure of 100 lb./in. absorption may continue at 14 per cent. of the original rate at atmospheric pressure. These results are in perfect agreement with those obtained in Series II (see summary of Series II in earlier communication (4), p. 233).

A notable feature of all the curves to the mean experimental results, (Table XIV, and Fig. 2) is that they always fall most sharply at first and

that the rate of reduction of the absorption falls off considerably with increase in the deficit. When a deficit is created, therefore, the absorption is immediately substantially reduced, nearly always falling off to half its original value by the time the deficit has reached the first 30–45 lb./in. (2–3 atm.). After this the curves fall less and less rapidly, evidently on account of the fact already mentioned above, that some adjustment is made by the plant which tends to reduce the effect of a given deficit. In other words, a time factor comes into play and the curve comes to fall less and less steeply as more and more time is allowed for the adjustment to take place. It is significant in this connexion that the more quickly the pressure is raised (and the fewer points there are on the graph) the more steeply does the curve fall (see Privet at two-minute intervals). Evidently if the pressure were raised very slowly the curves would fall very slowly also and given pressures would have less effect than when they are reached suddenly. The effect of a given deficit will therefore depend upon the rate at which it is obtained. If given pressures were maintained, moreover, instead of being further increased, it would appear that some recovery might be made towards the original absorption rate. These points are dealt with more fully in the next series (Series V).

SERIES V.

In the experiments of this series the effects of sustained constant deficits of 20–300 lb./in. (i.e. up to 20 atm.) were studied.

The results are given in Tables XV–XXI and Figs. 3, 4, and 5.

A constant rate of absorption was first of all obtained at atmospheric pressure. The pressure was then raised to a definite value decided beforehand (the actual raising of the pressure taking about half a minute), and then maintained constant for as long as it was possible to continue taking readings of the absorption rate, i.e. until all the protruding stem had been cut away for readings. The absorption rate was followed up over the whole period. The effects of decreasing the deficit after longer or shorter exposures were also determined in some cases (Tables XV, XVII, and XIX). The actual readings were taken always at regular intervals of time as indicated by the 'time' columns in the tables, the first being taken two minutes after beginning to raise the pressure. This procedure was adopted because in some cases (e.g. Table XIX, Expts. 2 and 7, &c.) the immediate effect of the deficit was not the maximum one, the percentage absorption rate falling off during the first two or three minutes of exposure.

In the case of the Privet two different procedures were adopted. In one set of experiments the absorption was followed up throughout the period of deficit as just described. These experiments are described as 'Long Experiments' in the tables (Table XIX). In other cases the

TABLE XV.

Results of Experiments on Acer showing the Relation between Absorption Rate and Time at Different Constant Pressures.

The rates of absorption are given also as percentages of the rates of absorption at atmospheric pressure determined before the higher pressures were applied.

Experiment 1.				Experiment 5.			
Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate as percent. of rate at atm. press.	Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate as percent. of rate at atm. press.
0	0	3.6	100	0	0	4.1	100
0	25	2.7	75	0	200	0.0	0
5	25	3.0	83.4	10	200	0.0	0
10	25	3.1	86	20	200	0.3	7.3
20	25	3.1	86	30	200	1.8	44
30	25	3.1	86	40	200	2.7	66
				51	200	2.75	67
				64	200	2.8	68
				80	200	2.5	61
				100	200	2.3	56
				130	200	2.0	49
				165	0	20	490 ¹
Experiment 2.				Experiment 6.			
0	0	2.5	100	0	0	4.3	100
0	50	1.7	68	0	250	0.0	0
5	50	1.8	72	10	250	0.0	0
10	50	1.9	76	20	250	0.0	0
25	50	1.95	78	30	250	0.0	0
40	50	2.0	80	40	250	0.0	0
55	50	2.0	80	50	250	0.0	0
70	50	2.0	80	60	250	0.0	0
85	50	2.0	80	70	250	0.0	0
				80	250	0.2	4.7
				90	250	0.6	14
				130	250	1.8	42
				145	250	1.9	44
				160	250	2.0	46
				175	250	2.1	49
				190	250	2.2	51
				205	250	2.3	53
				230	250	2.2	51
				250	0	5.0	116 ²
Experiment 3.				Experiment 4.			
0	0	4.1	100	0	0	3.7	100
0	100	2.5	61	0	150	0.0	0
5	100	2.6	63	5	150	0.0	0
15	100	2.8	68	15	150	1.0	27
25	100	3.0	73	25	150	2.5	68
35	100	3.1	76	35	150	2.7	73
60	100	3.1	76	45	150	2.5	68
				55	150	2.3	62
				65	150	2.2	59

¹ Plant still quite turgid on removal from cylinder.² Leaves found to be slightly flaccid on removal of plant from cylinder.

TABLE XVI.
(Collected Results from Table XV.)

Pressure.	Initial rate at increased pressure.	Maximum rate at increased pressure.
	%	%
25	75	86
50	68	80
100	61	76
150	0	73
200	0	68
250	0	53

(All rates as percentages of initial rate at atmospheric pressure.)

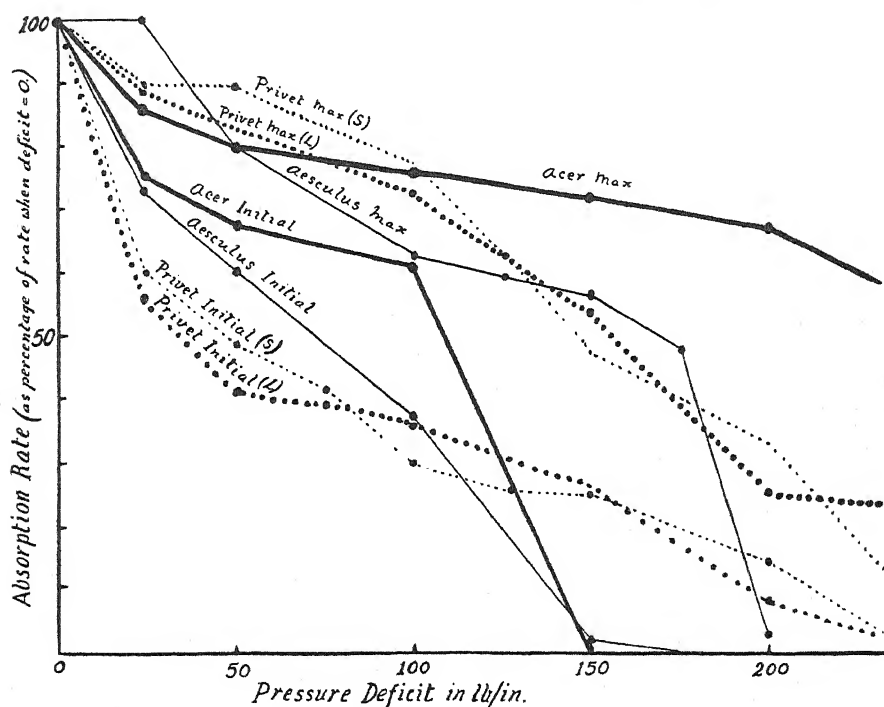


FIG. 3. Curves showing initial and final or minimum and maximum rates of absorption under different sustained constant deficits. The points are obtained from Tables XVI, XVIII and XXI. (s) indicates 'short' experiments; (L) indicates 'long' experiments. It is shown that the rates of transpiration under different deficits are given by the 'maximum' curves in this graph.

constant rate at atmospheric pressure and the initial rate under the deficit conditions were obtained in the same way and the pressure was then maintained constant for one hour without taking any readings. At the end of this time the 'final' rate was measured, since it will be seen from the 'Long' experiments that the maximum rate usually occurred after approximately one hour's exposure to the higher pressure. Experiments performed

TABLE XVII.

Results of Experiments on Aesculus showing the Relation between Absorption Rate and Time at Different Constant Pressures.

The rates of absorption are given as percentages of the rates of absorption at atmospheric pressure determined before the higher pressures were applied.

Experiment 1.				Experiment 5.			
Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate as percent. of initial rate.	Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate as percent. of initial rate.
0	0	2.2	100	0	0	3.0	100
0	25	1.6	73	0	150	0.05	1.7
5	25	1.9	86	5	150	0.1	3.3
10	25	2.1	96	15	150	0.8	27
15	25	2.2	100	25	150	1.5	50
20	25	2.2	100	40	150	1.6	53
25	25	2.2	100	55	150	1.7	57
27	0	ca 4.4	ca 200	65	150	1.7	57
				68	0	ca 2.1	ca 700
Experiment 2.				Experiment 6.			
0	0	3.0	100	0	0	3.3	100
0	50	1.8	60	0	175	0.0	0
3	50	1.9	63	5	175	0.0	0
14	50	2.2	73	10	175	0.0	0
24	50	2.3	77	15	175	0.0	0
34	50	2.4	80	20	175	0.05	1.5
44	50	2.4	80	25	175	0.1	3
54	50	2.4	80	30	175	0.2	6
55	0	ca 10	ca 330	35	175	0.6	18
Experiment 3.				40	175	1.2	36
0	0	2.4	100	45	175	1.6	48
0	100	0.9	37.3	50	175	1.6	48
2	100	1.0	41.5				
7	100	1.1	46				
17	100	1.5	62.5				
27	100	1.5	62.5				
37	100	1.5	62.5				
47	100	1.5	62.5				
49	0	5	550				
Experiment 4.				Experiment 7.			
0	0	2.5	100	0	0	2.0	100
0	125	0.0	0	0	200	0.0	0
5	125	0.0? +	0? +	5	200	0.0	0
10	125	0.0? +	0? +	15	200	0.0	0
15	125	0.7	28	30	200	0.0	0
20	125	1.1	44	45	200	+	+
25	125	1.3	52	70	200	0.05	2.5
30	125	1.5	60	72	0	9.6	480
47	125	1.5	60				
62	125	1.5	60				

TABLE XVIII.
(Collected Results from Table XVII.)

Pressure.	Initial rate at increased pressure.	Maximum rate at increased pressure.
	%.	%.
25	73	100
50	60	80
100	37.5	62.5
125	—	60
150	1.7	57
175	0	48
200	0	2.5

(All rates are given as percentages of the initial rate at atmospheric pressure.)

in this way without reading the intermediate values are referred to as 'Short' experiments (Table XX). It was hoped in this way to obtain more accurate relative values of the maximum rate under the deficit, but the results obtained by both methods were substantially the same (cf. Curves from short and long experiments, labelled S and L respectively in Fig. 3). Since, however, the branch was not all used up in obtaining intermediate readings it was possible in this type of experiment to investigate the effects of subsequent reduction of the deficit by different amounts (Table XX). This was also investigated in some experiments on *Acer* and *Aesculus* (Tables XV and XVII).

The actual experimental results of the whole series are given in Tables XV, XVII, XIX, and XX. The initial and final (i.e. minimum and maximum) values of the rates under pressures are collected in Tables XVI, XVIII, and XXI. Graphs of these values will be found in Fig. 3, and characteristic recovery curves are plotted for *Acer* in Fig. 4 (based on Table XV) and for *Aesculus* in Fig. 5 (based on Table XVII).

General Conclusions from Series V.

The general results of this series may be summarized as follows.

The establishment of a deficit leads to an immediate reduction in absorption rate to a percentage of the original value which depends upon the pressure applied. The higher the pressure the lower the percentage of the original rate (Tables XV, XVII, XIX, and XX).

The rate reaches its minimum value immediately in most cases, but sometimes only after a few seconds under the deficit (e.g. Table XIX, Expts. 2 and 7).

The reduction in rate is relatively greater for the smaller deficits, the earliest stages in the establishment of a deficit also making relatively the most difference to the absorption rate. (Tables XVI, XVIII, and XXI, and 'Initial' curves in Fig. 3: cf. Fig. 2).

TABLE XIX.

Results of 'Long'¹ Experiments on Privet (Ligustrum) Showing the Relation between Absorption Rate and Time at Different Constant Pressures.

The rates of absorption are given also as percentages of the rates of absorption at atmospheric pressure determined before the higher pressures were applied.

Experiment 1.				Experiment 5. ¹			
Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate %.	Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate %.
0	0	4.5	100	0	0	4.1	100
0	25	2.5	55.5	0	100	1.4	34.1
22	25	3.5	77.7	20	100	1.7	41.5
37	25	3.8	84	45	100	3.0	73.2
47	25	4.0	89	60	100	2.5	61
57	25	3.7	82	65	75	6.0	146.5
67	25	3.0	66.6	68	100	1.5	36.6
77	25	2.7	60	70	50	>10	>244
Experiment 2.				Experiment 6.			
0	0	4.2	100				
0	50	1.7	40.5				
5	50	1.5	36.7				
10	50	1.7	40.5				
15	50	2.1	50	0	0	4.8	100
20	50	2.6	62	0	100	1.8	37.5
25	50	2.8	68	2	100	1.8	37.5
30	50	3.0	71.4	15	100	1.9	39.5
45	50	3.5	83.3	20	100	2.0	41.6
55	50	3.5	83.3	25	100	2.3	48
Experiment 3.				35	100	3.1	64.5
0	0	4.0	100	45	100	3.5	72.8
0	75	1.6	40	55	100	3.4	71
2	75	1.6	40				
7	75	1.8	45				
12	75	2.0	50				
17	75	2.5	62.5				
27	75	3.0	75				
37	75	3.1	77.5	0	0	4.8	100
47	75	3.1	77.5	0	150	1.3	27
57	75	2.9	72.4	8	150	1.1	23
72	75	2.8	70	13	150	1.4	29
Experiment 4.				18	150	1.7	35
0	0	4.1	100	23	150	2.0	42
0	75	1.6	39	28	150	2.1	44
3	75	1.6	39	33	150	2.2	46
35	75	3.0	73	38	150	2.6	54
60	75	3.3	80	43	150	2.6	54
65	50	11.0	268	48	150	2.6	54
				58	150	2.5	52
				63	0	30	625

¹ See p. 543.

TABLE XIX (continued).

Experiment 8.				Experiment 9.			
Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate %.	Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate %.
0	0	7.5	100	0	0	6.0	100
0	200	0.6	8	0	250	0.0	0
5	200	0.9	12	5	250	0.0	0
10	200	1.3	17.3	10	250	0.0	0
15	200	1.5	20	15	250	0.0	0
20	200	1.7	22.7	20	250	0.0	0
30	200	1.8	24	25	250	1.0	16.6
40	200	1.9	25	35	250	1.3	21.7
50	200	1.9	25	45	250	1.4	23
60	200	1.9	25	55	250	1.4	23
				80	250	1.4	23
				100	250	1.4	23
				110	0	>50	>833

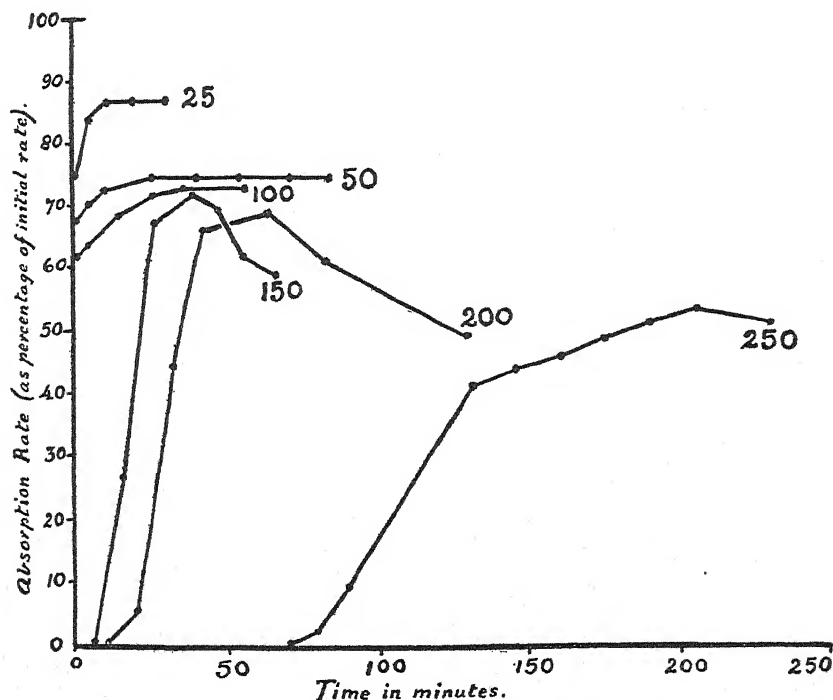


FIG. 4. Recovery curves for *Acer*. The curves show the increases in absorption rate with time under sustained constant deficits. Each curve shows the Rate-Time relation at one pressure. The pressures are indicated by the figures at the ends of the curves. (Table XV.) The absorption rates are all given as percentages of the rate under atmospheric pressure.

TABLE XX.

Results of 'Short'¹ Experiments on Privet (Ligustrum) Showing the Absorption Rates at Various Pressures (a) Immediately after the Increase of Pressure, and (b) One Hour after the Increase of Pressure.

The rates of absorption are given also as percentages of the initial rates of absorption at atmospheric pressure determined before the higher pressures were applied.

In some cases the experiments are extended to give the values of the absorption rates during a longer period of sustained pressure, the values during slight reduction of the pressure or the values resulting from the subsequent reduction of the pressure to atmospheric pressure.

Experiment 1.				Experiment 7.			
Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate as percent. of rate at atm. press.	Time from increase of pressure (minutes).	Press. in lb./in.	Rate of absn. obsd.	Rate as percent. of rate at atm. press.
0	0	6.5	100	0	0	4.0	100
0	25	3.5	54	0	100	1.2	30
60	25	5.0	77	60	100	3.1	77.5
63	25	5.5	84.5	63	150	0.5	12.5
66	25	5.8	89.3	66	100	10.5	262
69	25	5.5	84.5				
72	20	6.0	97.2				
Experiment 2.				Experiment 8.			
0	0	3.0	100	0	0	4.8	100
0	25	2.0	66	0	125	1.3	27
60	25	2.7	90	60	125	3.0	62.5
65	50	1.8	60	63	115	5.5	115
67	25	7.0	233	65	100	10.0	208
Experiment 3.				Experiment 9.			
0	0	4.3	100	0	0	3.8	100
0	50	2.0	46.5	0	150	1.0	26.3
60	50	4.3	100	0	150	1.8	47.5
65	50	4.3	100	60	150	1.8	47.5
68	40	7.2	167.5	65	100	11.0	290
Experiment 4.				Experiment 10.			
0	0	4.2	100	0	0	4.3	100
0	50	2.2	52.4	0	200	0.6	14
60	50	3.8	90	60	200	1.4	32.6
62	25	5.8	138	65	180	4.2	97.5
Experiment 5.				70	0	20	465
0	0	3.7	100				
0	50	1.8	49				
60	50	3.0	81				
Experiment 6.				Experiment 11.			
0	0	3.6	100	0	0	5.0	100
0	75	1.5	41.6	0	250	0.0	0
60	75	3.0	83.3	15	250	0.0	0
65	65	5.0	139	30	250	0.0	0
70	50	8.0	222	45	250	0.0	0
72	0	30	833	60	250	0.0	0
				65	200	16.0	320

¹ See p. 543.

TABLE XXI.

(Collected Results from Tables XIX and XX on Privet.)

All rates are given as percentages of the initial rates at atmospheric pressure read before the increased pressures were applied.

Pressure.	'Long'		'Short'	
	Experiments.		Experiments.	
	Initial rate at increased pressure.	Maximum rate at increased pressure.	Initial rate at increased pressure.	Maximum rate at increased pressure.
0	100	100	100	100
25	{ 55.5	89	54	89.3
	{ —	—	66	90
	{ 40.5	83.3	46.5	100
50	{ —	—	52.4	90
	{ —	—	49	81
	{ 40	77.5	41.6	83.3
75	{ 39	80	—	—
	{ 34.1	73.2	30	77.5
100	{ 37.5	72.8	—	—
125	—	—	27	62.5
150	27	54	26.3	47.5
200	8	25	14	32.6
250	0	23	0	0

With time the rate under a sustained constant deficit recovers more or less towards its original value. It increases again to a higher percentage of the original rate dependent upon the value of the deficit. (Tables XV, XVII, and XIX, and Figs. 4 and 5.)

The recovery or increase of rate with time begins immediately at lower pressures, but only at increasingly longer times after the beginning of the period of deficit at the highest pressures. (Tables XV, XVII, and XIX and Figs. 4 and 5.)

The initial rates (Tables XVI, XVIII, and XXI, and 'Initial' curves, Fig. 3) are high as compared with those of Series IV (Fig. 2) evidently owing to the allowance of the two-minute interval (p. 543) which allows slight recovery to take place. Moreover, they fall off much more sharply at or about the pressures corresponding to the original osmotic values of the leaf cells (cf. Osmotic values given under Tables VIII and IX). Those of Series IV (Fig. 2) fall off less and less rapidly with increasing deficit and are positive to much higher pressures. This is evidently because the same adjustment on the part of the leaf cells which allows of the recovery observed in Series V has been taking place to some extent during the periods required to raise the pressure in the experiments of Series IV.

The final values of absorption rate attained under pressure may be equal to the original values at the very lowest deficits (e.g. 1–2 atm.), but at higher deficits are progressively smaller percentages of the original rates.

The final or maximum rates attained under maintained deficits are plotted together with the initial rates obtained in the same experiments in Fig. 3 for all the different plants used. The results for the different plants are the

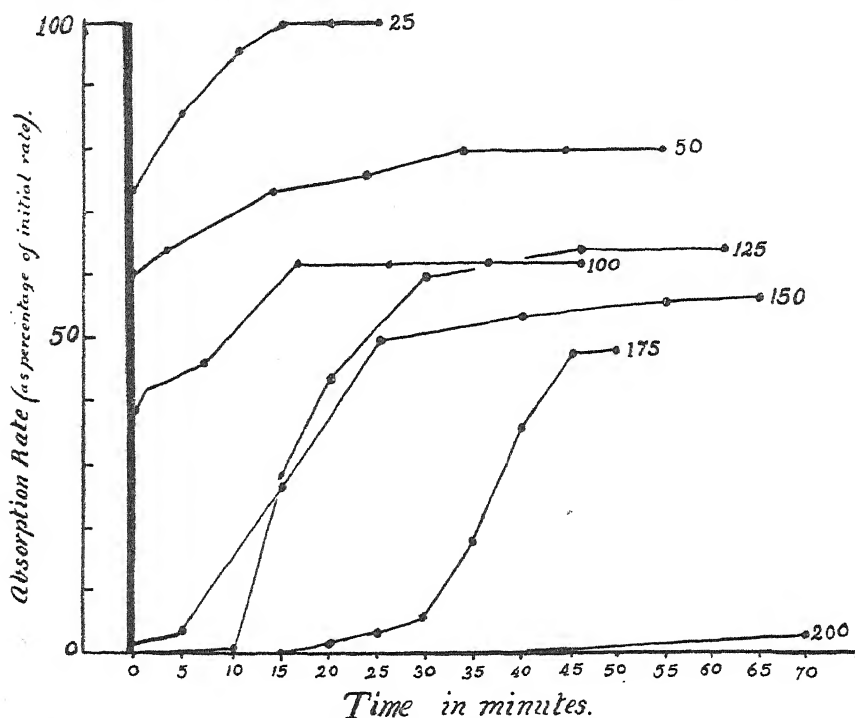


FIG. 5. Recovery curves for *Aesculus*. The curves show the increases in absorption rate with time under sustained constant deficits. Each curve shows the Rate-Time relation at one pressure. The pressures are indicated by the figures at the ends of the curves. (Table XVII.) The absorption rates are all given as percentages of the rate under atmospheric pressure.

same in general character and in the relative positions of the two curves, the curves to the final rates always running above those to the initial rates. The final rates, like the initial rates, fall off with increasing pressure, but it will be observed are always positive and frequently considerable even at pressures far above those causing reversal in Series IV, and far above those corresponding to the determined osmotic values of the leaf cells (Fig. 3). (Osmotic values in Tables VIII, IX.) The maximum rate may apparently only be sustained for long as a constant rate at the lower deficits (e.g. less than 100 lb./in.) and may fall off again if the deficit be large (Table XV, Expts. 4, 5, 6, and Table XIX, Expt. 7).

A reduction of the deficit always leads to an immediate and altogether disproportionate increase in the rate of absorption. This is well seen in Table XV, Expts. 5 and 6; Table XVII, Expts. 1, 2, 3, 5, and 7; Table XIX, Expts. 5, 7, and 9. Experiment 5 of Table XIX is interesting as

showing the effects of a second increase and decrease of the deficit. It will be seen that when a little recovery has been made at a reduced deficit (75 lb./in.), the effect of a further increase (to 100 lb./in.) is nearly as great as on the original material, the rate being reduced far below the last value at this pressure. This appears to be generally the case. All the results fall well into line with those obtained in Series II (see 4, Tables V–XI, and Fig. 3).

The results in terms of leaf-pull¹ might be summarized as follows.

Increase of deficit decreases leaf-pull and therefore conduction rate, the decrease being greatest at first. Successive increments in the deficit have progressively less effect on the leaf-pull until a final more rapid falling off takes place.

At a certain deficit the leaf-pull becomes zero: at higher values it is negative and the stem draws water back from the leaf.

The value of leaf-pull immediately resulting from a given deficit is a definite percentage of the original leaf-pull. If, however, the deficit be sustained the leaf-pull increases again to a definite higher percentage of the original value. The lower the deficit the more complete is the recovery and the more quickly does it take place.

Decrease in deficit leads to an enormous increase in leaf-pull, which later falls back to its original value.

Theoretical Interpretation of the Results.

It now remains to be seen what conclusions can be drawn from the conduction rates which have been measured concerning the rates of transpiration under different pressures which it was required to find. It is obvious that the conduction rates measured will be equal to the rates of transpiration only at such times as no changes in volume or water content are taking place on the part of the leaf cells: that is to say, when the leaf cells have attained volumes and osmotic pressures which are in complete dynamic equilibrium with the deficit and the rates of transpiration and conduction which take place under it. At other times, that is to say, during the attainment of a new equilibrium when a change of deficit has taken or is taking place, the conduction rates will be smaller than the transpiration rates when the pressure deficit is increasing and the leaf cell volumes decreasing; and greater than the transpiration rate when the pressure deficit is falling and leaf cells re-expanding. It will be useful to consider the original state of equilibrium in detail and the effects upon this equilibrium when a deficit is applied. Reference may be made to Fig. 1 of the earlier paper (4, p. 216).

¹ Inasmuch as the tensions in the stem tracts in nature must exist also in those of the leaves, the term 'leaf-pull' is used here to indicate the total force developed in the leaves and tending to raise water, or the balance between the leaf cell suction pressure and the leaf tract tension, &c., and not the positive forces alone. In the experiments also, it implies the balance of *all* forces at work in the leaves and not only the leaf cell suction pressure.

Let P be the osmotic pressure of the leaf cells,
 p , the osmotic pressure of the contents of the tracts,
 T , the turgour pressure of the leaf cells,
 Q , the pressure at the leaf surfaces, and
 q , the hydrostatic pressure in the tracts.

Then, as already shown (4, p. 217), the force tending to move water upwards = $P + q$, and the force tending to move water down = $p + T + Q$.

The osmotic pressure of the water supplied to the tracts in the experiments, p , is negligible, hence, at equilibrium

$$P + q = T + Q \text{ or } P - T = Q - q$$

or suction pressure of leaf cells = pressure deficit.¹ When the deficit, $Q - q = 0$, the equilibrium suction pressure, $P - T = 0$.

Let it be supposed that the experimental material be initially saturated and fully turgid. The turgour pressure of the leaf cells is then equal to the osmotic pressure and $P - T = 0$. The leaf cells are therefore in equilibrium with a deficit of zero (no applied pressure).

When excess pressure is applied (deficit created) new values of leaf cell volume and osmotic pressure and therefore of suction pressure must be attained such that another similar equilibrium can be established. The new suction pressure must balance or equal the deficit, or $P' - T' = Q - q$, where P' and T' are the new values of the osmotic pressure and the turgour pressure. P' will be slightly greater than P , and T' will be considerably less than T . This change involves loss of water by the cell: This water has to pass out through the plasmatic membranes and the walls and therefore the new equilibrium takes time to become complete. When the deficit is first created or the excess pressure first applied, the forces causing water to flow out of the cell will be the new value of Q (the applied pressure) + the original value of T (the turgour pressure) acting together. Thus the initial force driving water out of the cell = $Q + T$. As the change takes place, however, the cell diminishes in volume and the value of T becomes considerably reduced. When the new equilibrium is established such that $P' - T' = (Q - q)$, since P' will be only very slightly greater than P , and $P' = (Q - q) + T'$, and $P = T (Q - q) + T'$ will be only very slightly greater than T . In other words, the excess pressure is not added to the existing turgour pressure but replaces it. The applied pressure, however, can only relieve the turgour pressure in this way by causing slight compression of the cell which reduces the extent of stretching of the walls and therefore the tension which causes the turgour pressure. The effect of the applied pressure may be compared with that of the outer tyre of a motor-car in relieving the tension on the inner tube. At first the force making for back-

¹ Allowance for intercellular pressure does not affect the argument, so is omitted for simplicity.

flow is $Q + T$ but it falls off to $Q + T'$ as equilibrium is reached. These considerations go far to explain the reversals noticed on sudden increases of Q to values below the osmotic value with turgid cells (above, pp. 541-3) and also the phenomenon of recovery as far as the forces responsible are concerned. The force opposing absorption falls off with time under the deficit as the original wall tension is relieved and disappears.

We have so far, however, only considered static equilibria. In practice transpiration is also proceeding. The loss of water causes an increase of P and a decrease of T . $P - T$ becomes greater and the cells slightly smaller. This increases the lifting force or leaf-pull until values of the leaf cell volume and $P - T$ are reached which are in dynamic equilibrium with the rate of transpiration and the resistance to flow. This value of $P - T$ is maintained somewhat above the static equilibrium value, the excess over the latter being the effect of the transpiration rate and the cause of the conduction rate. The increase of $(P - T)$ would be expected also to reduce the transpiration rate somewhat by reducing the water supply to the leaf cell walls from the cell interiors and therefore reducing the vapour pressure at the cell surfaces. Dynamic equilibrium is therefore attained when the value of $P - T$ has become sufficiently great to cause a conduction rate against the resistance to flow, R , equal to the rate of transpiration. At this point,

$$\text{Transpiration Rate} = \text{Conduction Rate} = C = \frac{(P - T - q) - (p - q)}{R}.$$

The osmotic pressure of the tract contents, p , however, is negligibly small compared with the other quantities and may be omitted, so that more simply

$$C = (P - T)/R.$$

When excess pressure is applied, just as in the case of static equilibria already considered, the deficit is first added to the existing downward forces, so that

$$C = \frac{(P - T) - (Q - q)}{R}.$$

Consequently the conduction rate will be considerably reduced or even reversed if the deficit $(Q - q)$ be greater than the suction pressure $(P - T)$. As the new equilibrium is attained, however, just as in the case of static equilibria the excess pressure comes to replace the pre-existing turgour pressure at least in part. The leaf cell volume decreases, the turgour pressure is reduced and new values of the volume, the osmotic pressure and the turgour pressures are reached in equilibrium with the newly imposed deficit and the transpiration rate. This will be such that the total pull is again just capable of causing a conduction rate against the (possibly new) value of R (see below, p. 561) equal to the (probably new) value of the transpiration rate.

$$\text{Then Transpiration rate} = \text{Conduction rate} = \frac{(P' - T') - (Q - q)}{R}.$$

Neglecting p , therefore, the conduction rate measured is always given by

$$C = \frac{\text{Suction pressure} - \text{Deficit}}{\text{Resistance}}.$$

The excess of the suction pressure over the deficit (i.e. over the static equilibrium value) which causes the flow depends in turn upon the transpiration rate, the conduction becoming constant and equal to the transpiration rate when the latter is sufficient to maintain the necessary excess of the suction pressure over the deficit to cause a rate of conduction equal to itself.

It is evident from these considerations that the relatively large effects of small pressure deficits when first applied, the recovery effects with all pressures and the disproportionate increases in absorption on decreases of the deficit which have been recorded might be explained entirely in terms of changes in the volumes of the leaf cells. The large effects of small pressures would thus be due to the temporary working together of the original turgour pressure and the newly applied pressure during the attainment of the new equilibrium (p. 554), causing a rapid decrease in cell volume. The recovery effects would be due to the gradual increase of suction pressure and therefore of lifting force as the cells attained their new (smaller) equilibrium volumes, and the enormous rates of conduction which follow reductions in the deficit would be interpreted as due to the re-expansion of the mechanically compressed cells.

There are, however, other possibilities.

Thus it is evident from the equations given above (p. 555), that the conduction rate would be altered by any induced secondary change in the osmotic pressure of the leaf cells through activation or inactivation of osmotic substances which might be induced by the deficit, as this would alter the suction pressure. In addition to this the strain of the deficit might affect the cells in some way capable of causing alterations in the value of the resistance to flow through the protoplasmic membranes or even through the walls, which would also have its effect upon the conduction rate.

The variations noted may therefore be accounted for by some or any of the following factors:

- (1) Changes in the rate of transpiration,
- (2) Changes in the degree of mechanical compression of the cells,
- (3) Secondary changes in osmotic pressure of the leaf cells, and/or
- (4) Changes in the value of the resistance to flow.

The immediate effects of changes in the deficit must be due to (1) and/or (2) and just possibly (4) since there would scarcely be time for (3) to come into play. Recovery under sustained deficits and the increase on reduction of the deficit might, on the other hand, be in part due to altera-

tions in P or R or both. Thus recovery might be due to a steady increase of the leaf cell osmotic pressure.

The possibility of accommodating secondary changes in the osmotic pressures of the leaf cells under deficits appeared to be of particular interest, since if any such changes took place the phenomenon might afford a mechanism whereby the bubbles which occur from time to time (5) in conducting tracts could be forced back into solution. Thus if increase of deficit caused an increase in the leaf cell osmotic pressure it would be natural to suppose also that a decrease in the deficit would lead to a decrease in the osmotic pressure by reversal of the same process. This would mean that the decrease of deficit at night in natural conditions might lead to a reduction of the leaf-pull in such a way that the leaf cells lost some of their water to the stem and allowed the water columns in the tracts, so to speak, to rest back for a while instead of being supported by cohesion from the top. The pressure in the tracts would then become positive for a time until the reactivation of the leaf cell solutes set in and bubbles in the tracts would meanwhile disappear. Unfortunately for this tempting hypothesis, however, the evidence goes to show (p. 559) that there is no change whatever of the sort required in the leaf cell osmotic pressure of any of the plants investigated at any deficit, so the results shed no light upon the mechanism of the disappearance of bubbles from the tracts at night. The suggestion may, however, be worth following up in other directions.

The parts played by the different possible factors (1), (2), (3), and (4), above (p. 556), will be discussed in the light of some further relevant experiments which will be described in Series VI. In the experiments of this series the absorption rates at different deficits and following different changes in the deficit were measured as in the preceding series, but with branches which had been thoroughly vaselined all over, thus eliminating effects due to changes in transpiration rate altogether. That the changes in absorption rate are partly due to changes in the degree of mechanical compression of the cells and to their re-expansion is already evident from the results of Series II (4, p. 233), the different effects of a given pressure according to the previous history of the material being evidently to be explained in terms of the different initial degrees of compression of the cells. All the facts in the summary of Series II could evidently be interpreted in this way (4, p. 233). The following experiments, with branches in which transpiration was entirely prevented by vaselining, bear out the same conclusion.

SERIES VI.

Pressure Cylinder Experiments with Vaselined Branches.

Experiment 1. Aesculus. Simple potometer method.

Leaves and stems and petioles all thoroughly vaselined.

Initial rate of absorption when completely saturated = 0.

At pressure of 50 lb./in. water was exuded from the cut end of the stem into the potometer at a rate of 100 divs./hour. This continued for half an hour.

Pressure then raised to 100 lb./in. The rate of exudation increased. On returning to atm. it was observed that water was forced out of the stem for 6 minutes after the pressure had been completely released. This effect is evidently due to air in the intercellular spaces remaining under compression for some time after release owing to the time required for its outward flow through the stomata.

Seven minutes after the release of the pressure, absorption started again very slowly (at 0.25 divs./min.) and continued steadily for 10 minutes.

A momentary increase of the pressure to 70 lb./in. again produced a negative flow at a rate of -5 divs./min. On release of the pressure this rapidly slackened to 0, re-absorption at a rate of 3.3 divs./min. being established within 5 minutes. This rate gradually fell off, but absorption was still continuing $1\frac{1}{2}$ hours later at a rate of 1.88 divs./min.

The experiment shows changes in the conduction rate which can only be due to compression and re-expansion of leaf cells. The mechanical expression of water from the leaf cells into the tracts evidently begins before a pressure is reached which is equivalent to the osmotic pressure of the leaf cells at least when the pressure is applied suddenly (as already predicted, p. 555) though not necessarily when it is raised gradually, as the osmotic pressure of the leaf parenchyma cells determined plasmolytically by means of glucose solutions varied between 7 and 12 atmospheres. The explanation of this is given above (p. 554). In experiments with increasing pressure deficits then the mechanical effect may lead to rates of absorption which are low as compared with the current rates of transpiration, but when the pressure deficit is reduced it is important to note that the mechanically induced exudation continues *after* the reduction in pressure has ceased. The effect of the reduction of the deficit on the transpiration rate must, therefore, be actually *greater* than that indicated by the change in the rate of absorption. Since reduction of the deficit has therefore always an effect on the transpiration rate at least as great as that indicated by the change in the rate of absorption, it appears at least highly probable and reasonable to assume that increases in the deficit have equally great effects upon the actual transpiration rate in the opposite direction. The effects indicated in the foregoing experiments (Series II, III, IV, and V) are, therefore, not to be attributed entirely to the mechanical effects of compression and release of the leaf cells, but must at least in part be due to effects of the changes in pressure deficit on the rate of transpiration. (See pp. 559-60). Further evidence of this will be forthcoming later.

The above experiment serves mainly to emphasize the part played by the compression of leaf cells in decreasing the rate of absorption or causing exudation. The following experiment should also be quoted as emphasizing more particularly the part played by subsequent expansion of the cells in increasing the absorption rate.

Experiment 2. Aesculus. Simple potometer method.
Leaves and stems all thoroughly vaselined as in Expt. 1.
Initial rate of absorption by saturated branch = 0.

The pressure was raised to 100 lb./in. Exudation of water took place. In the course of 1 hour approximately 0.5 c.c. of water was expelled.

The pressure was then gradually reduced. After 5 minutes it had fallen to 55 lb./in., and at this point the meniscus became stationary and exudation ceased. After 1 minute more, when the pressure had fallen to 50 lb./in., a slow re-absorption had begun. The absorption rate then increased rapidly as the pressure fell, the rate 1 minute later being 1.54 and 2 minutes later (by which time the pressure had fallen to 0), 13.3.

Since rapid absorption recommences with thoroughly vaselined branches while still under pressure when the pressure is falling off it is definite that part at least of the increased absorption rate on release of the pressure in the experiments of Series II, IV, and V is due to re-expansion of leaf parenchyma cells. (See also 3, p. 1060).

In addition to the above preliminary experiments vaselined branch experiments were also carried out systematically on Aesculus, Acer, &c., according to the same procedure as in the experiments of Series V. A constant absorption rate was first obtained at atmospheric pressure and the pressure was then raised and maintained constant for one to two hours. Different pressures were used in the different experiments (cf. p. 543). These experiments showed conclusively that with constant pressures up to 100 lb./in. there was no recovery effect whatever. In no case was there any resumption of absorption whatever by vaselined branches under constant pressure. Rapid re-absorption, however, took place in the usual way on release or reduction of the pressure.

This series of experiments shows that there is therefore definitely *no* accommodation of the leaf cells to the pressure by increase of their osmotic pressures. Once the cells have contracted under the pressure they are *not* capable of any re-expansion until the pressure is again lowered. The apparent resumption of absorption or 'recovery effect' observed in Series V must therefore be due to an absorption rate dependent upon the transpiration which has been present all the time, but temporarily masked by the tendency towards expression of water from the leaves into the stems by compression of the leaf cells. Recovery is then due to a superposition of the temporary contraction effect which ends when the leaf cells have attained their equilibrium volumes upon the continuous absorption due to transpiration.

The effect is best illustrated by reference to Fig. 6.

It is evident from these considerations and from Fig. 6 that the transpiration rates characteristic of the different pressure deficits are given by

the rates of absorption when these latter have become constant and the preliminary compression effects have disappeared.

The rates of transpiration at different deficits are therefore given by

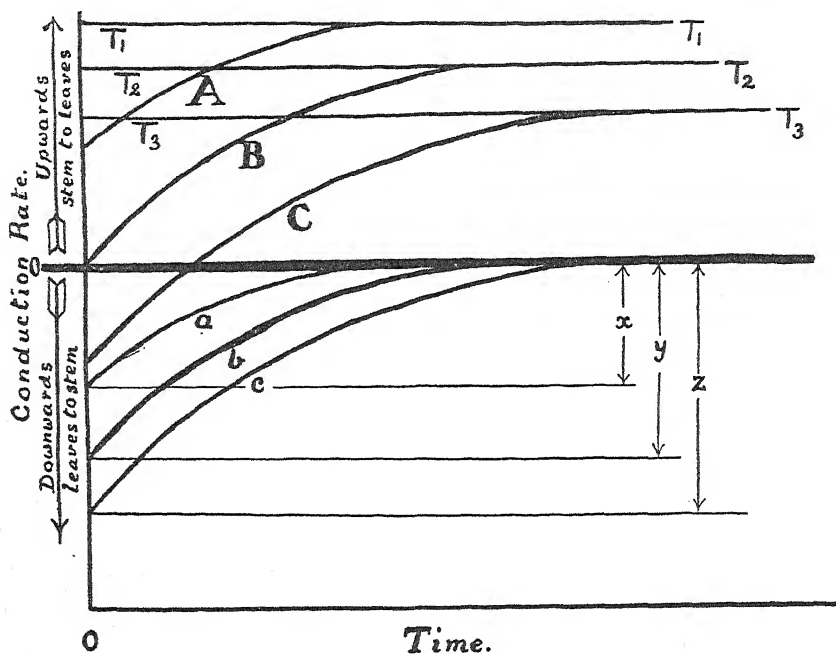


FIG. 6. The figure illustrates the mechanism of the recovery effect. Rate of flow (conduction rate) is plotted against time, points above the thick horizontal line (0) indicating flow upwards and points below it flow downwards. At the point 0 on the time axis where the curves start the pressure is raised to a constant value above atmospheric. At low pressure: let the transpiration rate be represented by T_1 . This will cause an upward flow represented by the curve T_1T_1 . If the back-flow due to compression start at a value, x , and fall off back to zero as the cells attain their equilibrium volumes, the back-flow rates will be given by the curve a . The resulting flow will therefore be given by subtracting curve a from the curve T_1T_1 which gives the curve A. At higher pressure: let the transpiration rate be T_2 , causing an upward flow represented by T_2T_2 . The back-flow will now be greater, $= y$ say, and as it falls off with time it will follow the curve b . The resulting flow will be given by subtracting this curve from the curve T_2T_2 , which gives the curve B. At still higher pressure: let the transpiration be T_3 , causing an upward flow represented by T_3T_3 . The back-flow will now be greater still, $= z$ say, and will fall back to zero with time according to the curve c . Subtracting this from the transpiration curve T_3T_3 , will give curve C. Thus at lowest pressures, (A), the absorption starts at a reduced value and increases to a constant one. At higher pressures, (B), it starts at 0 and increases to a constant value; while at still higher pressures, (C), it may begin negative (i.e. with reversed flow); the negative flow falls off and eventually a constant positive flow is established. In all cases there is 'recovery' and the constant value reached represents the transpiration rate alone. Whether or not reversal takes place depends upon the relation between the transpiration rate and the back-flow effect due to compression of the cells to their new equilibrium volumes.

the graphs (Fig. 3) showing the maximum absorption rates in the recovery experiments of Series V. The figures on which these are based are the figures for maximum absorption rates given in Tables XVI, XVIII, and XXI.

It is evident from the figures and from the graphs (Fig. 3, 'Maximum'

curves) that transpiration rate falls off considerably with increasing deficit. The rate of transpiration in all cases decreases most rapidly with the deficit at first. After this, in the case of the Privet, the relation appears to be more or less linear throughout, while in the other cases there is first a long period during which the transpiration rate falls off less rapidly with deficit, followed finally by a more rapid fall to zero. These general conclusions have been corroborated lately by a completely different method of direct measurement of the transpiration rates under pressure, using a thermopile. The results of these experiments will be discussed in a later communication.

The Resistance to Flow.

There remains for discussion the effect of the deficit, if any, upon the resistance to flow.

It must first be noted that although the analogy with Ohm's Law in electricity serves the purpose for which it has been used above (pp. 531, 555) it is not, strictly speaking, by any means true except for values at one rate of flow. It is true in electricity because the resistance of a given conductor is the same whether a small current be passed through it or a heavier one.¹ In the case of the branch, however, the resistance is due to viscosity, and will therefore increase very rapidly with the rate of flow. Thus, although $C = F/R$ will be true for one rate of flow, as the rate, C , increases the resistance will go up in proportion to a power of C , or $R \propto C^n$. Thus $C = F/C^n$, or the lifting force causing the conduction will be proportional to C^{n+1} . Thus, even if N be only $= 2$, which is probably the least value it could have, the force causing conduction (or the leaf-pull) would have to be proportional to the cube of the conduction rate. This force, as pointed out above (p. 556), will be the suction pressure of the leaf cells less the deficit, and will therefore be proportional to distances measured to the left from A along the x axis in Fig. 7, which illustrates a hypothetical rate-deficit curve, where A is the point at which the deficit becomes equal to the suction pressure, and the force and conduction rate therefore become 0. If there were no other effect upon the resistance besides its natural increase with rate of flow, then the conduction rate, C , should be connected with deficit by a curve of the type shown in which $A - x = C^3$, x being any value of the deficit. This curve is obviously of totally different type from those obtained experimentally, and the difference is evidently to be accounted for on the assumption that the deficit has an effect upon the protoplasts (or possibly also on the cell walls) whereby the value of R is increased. With values of the resistance increased by the deficits the intermediate values of the conduction rate would fall below those on the hypothetical curve. This they do in the experimental graphs, and it is

¹ Ignoring heating effects, &c.

evident, therefore, that the conditions of deficit increase the resistance of the leaf cells to flow. Such a suggestion has already been made by Köckemann (6).

CONCLUSIONS ON THE EFFECTS OF DEFICITS IN NATURE.

The inference from the general results for *Acer* and *Aesculus* is that in nature the gradual production of a deficit would lead at first to a considerable falling off in the transpiration rate, but that once a deficit corresponding to approximately a quarter of the original osmotic value of the leaf cells was reached there would be relatively little further change in the transpiration rate for a considerable period. Finally, a more rapid falling off would set in. The point at which this later more or less rapid fall would take place evidently depends upon the original osmotic values of the leaf cells and the extent to which they can be compressed without injury, i.e. upon the maximum suction pressure obtainable by undamaged cells. In the case of the Privet the fall in transpiration rate would be more regular and remain more or less linear against the deficit throughout. Owing to an increase in the resistance to flow the transpiration rate in all cases falls off more rapidly than the leaf-pull.

With very rapidly increasing deficits the conduction rate might fall off as rapidly as the absorption in the experiments of Series IV (Fig. 2), but it is very doubtful whether the deficit could ever increase as rapidly as this in nature. In natural circumstances it is to be noted that it is the withdrawal of water by the leaf cells which causes the pressure deficit in the tracts, and it is therefore impossible for a supra-equilibrium value of the turgour pressure ever to act in conjunction with the deficit in reducing the conduction rate (p. 554). The natural curves could therefore never fall quite as sharply as the experimental curves. Experiments with much more gradually increasing deficits have yet to be attempted. With more slowly increasing deficits a considerable period might elapse after the initial reduction, especially in the case of *Acer* (Fig. 3), during which the conduction and transpiration rates were but little further affected. In nature this would tend to cause the deficit to increase all the more rapidly, but even so, a relatively large volume of water would be lost before the final rapid fall in transpiration rate set in. A plant such as *Acer* with this type of maximum absorption rate-deficit curve would presumably be the least well fitted to withstand drought. The lower this curve the more resistant would be the plant, since the rate of water loss would be more reduced by a given deficit. Drought resistance would also naturally depend upon the rate at which a given deficit was produced under given conditions, but the extent to which the existence of a given deficit cut down the rate of water loss (cf. 2, p. 824), would undoubtedly also be an important factor deciding drought resistivity, and incidentally one which

could be measured. In the case of *Aesculus* the effects would be very similar to those in the case of *Acer*. The initial decrease in transpiration appears not to set in immediately here, but when it does set in it is more

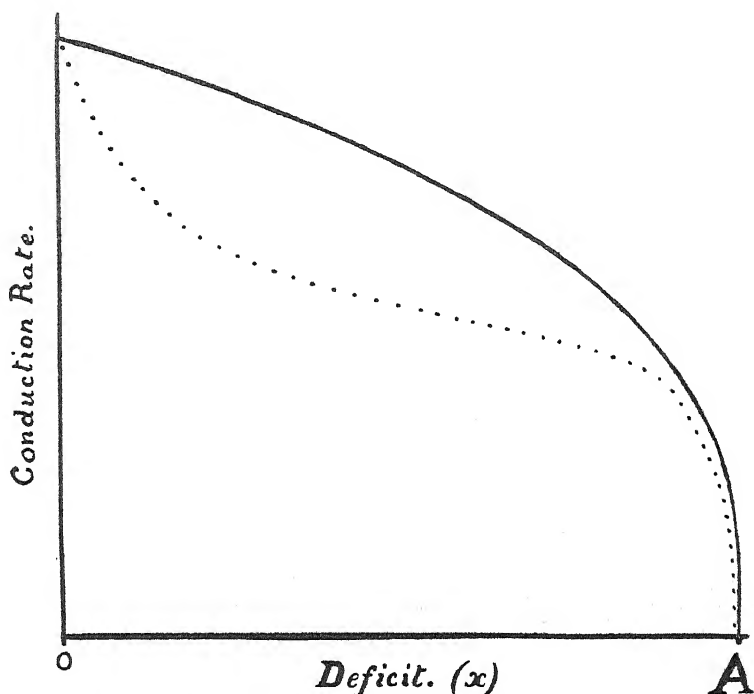


FIG. 7. The continuous curve shows the relation which would be expected to exist between conduction rate, C , and pressure deficit, x , on the assumption that the only changes taking place in the resistance to flow would be those naturally accompanying different rates of flow as a result of the fact that the resistance is due to the viscosity of a flowing liquid. The curve is of the form, $A - x = C^n$, where A is the value of the deficit at which it becomes equal to the suction pressure and the conduction rate becomes zero, x is the deficit, $A - x$ is therefore the moving force causing conduction, C is the rate of conduction and n in the curve plotted = 3. The dotted curve indicates the type of relation which is found by experiment. The difference is interpreted as due to an increase of the resistance to flow as a result of the deficit. This action on the resistance evidently becomes less as the point of more rapid falling off of transpiration or conduction rate is reached.

rapid at first, becomes less for a time, and falls off more rapidly again later when a deficit of about eleven atmospheres is reached. In the case of the Privet there would be a more or less regular fall throughout, the transpiration rate being reduced in linear relation to the increasing deficit. It will be noted that all forms dealt with happen to show a reduction in the rate of water loss to roughly 80 per cent. of the original rate by a deficit of 50 lb./in. in the experiments, though the reduction would not be expected to be quite as great in nature. It would be interesting to determine these values for definitely xeromorphic plants for comparison. It is hoped that further experiments may be possible in these directions later.

SUMMARY.

The rates of absorption by cut branches of *Acer*, *Aesculus*, &c., the upper parts of which are enclosed in a pressure cylinder, have been measured under different pressures by measurement of the rates of penetration of eosin solution.

With increasing pressures in the cylinder the absorption rate falls off with pressure. The decrease is most rapid at first and later less rapid, the absorption eventually ceasing and the direction of flow becoming reversed.

Reduction of the pressure leads to an enormous increase in the rate of absorption.

At constant pressures above atmospheric the rate of absorption is reduced considerably at first, but 'recovers' towards the original rate. Recovery is due to the cessation with time of the mechanical compression of the leaf cells which makes for downward flow of water until the cells have attained their new (reduced) equilibrium volumes under the increased pressure. There is no re-expansion of the cells before the pressure is again lowered, and no increase in osmotic pressure beyond that necessarily accompanying the decrease in volume.

After half to one hour under constant pressure the absorption rate becomes constant at a level depending upon the pressure used. The higher the pressure the lower is the percentage of the original rate attained.

It is shown that the final constant rates thus obtained under different pressures are measures of the rates of transpiration under these pressures. Thus transpiration rate can be plotted against pressure.

Increase of pressure deficit is thus found to decrease the transpiration rate, the reduction being relatively most marked at lower deficits. After the first reduction the transpiration is for a time less affected by further increase of the deficit until a final rapid falling off of the transpiration sets in.

It is shown that the deficit also increases the resistance to flow which is offered by the walls or the protoplasts of the leaf cells.

A fallacy in the application of Ohm's Law to problems of conduction is pointed out.

The bearing of the results on the effects of deficits in nature and upon drought resistivity is briefly discussed.

The cost of the apparatus with which this work was performed was defrayed by a grant from The Royal Society, to whom the writer is therefore much indebted.

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The Effect of the Rate of Flow of Air upon Assimilation and of Fluids upon Other Natural Processes.

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With one Figure in the Text.

DURING the course of experiments on hop drying, carried out under the auspices of The Institute of Brewing, I have *inter alia* investigated the effect of air velocity upon the time required for drying this material. The drying process is carried out by placing the hop cones upon a loosely woven horse-hair cloth, supported on an open slatted floor, and passing heated air upwards through the bed of hops. In commercial practice the depth of hops on the hair cloth varies from about 8 in. to about 30 in. The air in passing through the hops imparts heat to them and absorbs and carries away water in the form of vapour. The velocity of the air is therefore one of the factors which determine the length of time required for drying.

The air passing through the hops is at no time during the process saturated with water vapour; it is, in fact, during the greater part of the time far removed from this condition and is easily able to absorb any water vapour which it receives from the hops. The rate of drying, therefore, appears to be limited by the rate at which moisture diffuses from the hops to the air. The 'moisture deficit' of the air influences the rate of drying, as shown by the formula below, but in practice it does not become a limiting factor.

It has been shown by Langmuir and others (6, 7) that a stagnant film of fluid (or a layer of fluid in slow laminar movement) exists at the boundary surface of a fluid in motion. In the case of hop drying, water vapour from the hops has to traverse this more or less stagnant film of air before it can enter the main body of air moving around the hops, and this transfer can take place only by diffusion. The velocity of the air influences the thickness of the film; the higher the velocity the less thick will be the film and, consequently, the more rapidly will diffusion take place through it, other conditions being the same, for the less thick the film is the steeper will be the concentration gradient of the water vapour from that at the hop surface to that in the main body of air.

Other natural processes depend upon diffusion from or into a fluid, and in these also the diffusion takes place across the stagnant, or nearly stagnant, film of fluid in contact with the substance into or from which diffusion is taking place. Examples of such processes are the absorption of CO_2 from air by solutions of caustic alkalis and by green leaves during photosynthesis, and the solution of solids in liquids. It will be shown below how closely these processes agree with regard to the effect of fluid velocity upon the rate of drying, absorption, and solution respectively.

HOP DRYING.

Hops, which when fresh contain about 80 per cent. of moisture, are allowed to remain in the drying kiln until the average moisture content of the load is reduced to about 6 per cent. The time required for the hops to reach this state depends upon the depth of loading, temperature of the air, velocity of the air current through the hops, and atmospheric humidity. As a result of experiments on the subject (2, 3) I have derived the following formula by which the time required to dry a kiln-load of hops can be calculated :

$$T = \frac{1}{V.P. - v.p.} \left(\frac{716.5 L}{a^{1.047}} + \frac{6260}{a^{0.39}} \right).$$

T is the time (minutes) required for drying; $V.P.$ the vapour pressure of water (in. Hg) at the temperature of the air used in drying; $v.p.$ the vapour pressure of water already present in the atmosphere¹; L the loss in weight of the hops during drying, in ounces per square foot of kiln floor²; and a the air velocity (feet per min.) measured above the hops.

The second term of this formula, $\frac{6260}{(V.P. - v.p.) a^{0.39}}$, represents the minimum time which would be required to dry an infinitely shallow layer of hops under the given conditions. The first term, $\frac{716.5 L}{(V.P. - v.p.) a^{1.047}}$, represents the time taken for the level of dryness to proceed from the bottom of the load to the upper surface; this time is directly proportional to the depth of the load of hops on the kiln and inversely proportional to the air velocity.³

¹ If the vapour pressures $V.P.$ and $v.p.$ are measured in mm. of mercury the formula becomes :—

$$T = \frac{1}{V.P. - v.p.} \left(\frac{18200 L}{a^{1.047}} + \frac{159000}{a^{0.39}} \right).$$

² The loss of weight during drying is approximately 5 oz. per square foot per inch depth of green hops.

³ A certain contraction takes place, due to fall in temperature as the air passes through the hops, which causes the velocity to be lower above the hops than it is below. If the actual average air velocity within the bed of hops were taken $a^{1.047}$ would become a . $a^{0.39}$, however, would remain the same, as it is a factor of the minimum time required to dry an infinitely shallow layer of hops, in passing through which the air would suffer no contraction.

The fundamental effect of air velocity upon the time of drying is its influence on the minimum time, which is given by the second term of the formula; this is inversely proportional to the velocity to the power 0.39. This exponent of the air velocity appears to be independent of temperature over the range covered by the experiments, viz. 40° C. to 100° C.

ABSORPTION OF CO₂ FROM AIR.

(1) *Absorption by Caustic Alkalis.*

A process of diffusion across a stagnant, or slowly moving, layer of air similar to that which takes place during drying presumably occurs when CO₂ is absorbed by solutions of caustic alkalis, or by green leaves in photosynthesis. Consideration of this similarity led me to examine the results obtained by workers on the subject.

Brown and Escombe (1) in 1900, as a result of their investigations, state 'when the air current has attained a mean velocity of something like 300 metres per hour a further increase has no practical influence on the absorption', i.e. of CO₂ by solutions of caustic alkali. They give only three results of experiments with definite air velocities; they are shown in Table I below.

TABLE I.

Mean temperature °C.	Litres of air per hr. (at N.T.P.).	Mean velocity of air (m. per hr.).	Mean content of air in CO ₂ (parts in 10,000).	CO ₂ absorbed per sq. cm. per hr. (c.c. at N.T.P.).	
				Observed.	Corrected to 3 parts in 10,000.
14.4	91.9	156	2.57	0.127	0.148
15.0	182.6	311	2.86	0.165	0.172
14.5	295.2	503	3.07	0.182	0.177

If absorption of CO₂ from the moving air current were proportional to the 0.39 power of the air velocity the product of this and the time required to absorb any specified amount of CO₂ would give a constant number. In Table II the results which Brown and Escombe obtained are treated in this way:

TABLE II.

Air velocity (m. per hr.).	Time (min.) to absorb 1 c.c. CO ₂ (N.T.P.) per sq. cm.	Time × (air velocity) ^{0.39} .
156	405.5	2905
311	349.0	3273
503	339.1	3840

The product of time × (velocity)^{0.39} shows a rather wide variation and indicates that, in this case, the absorption increases somewhat more slowly

than would be the case if it were proportional to the air velocity to the 0.39 power.

Deneke (4) in 1931 made experiments on the rate of absorption of CO_2 by alkaline solutions; he circulated a limited volume of air at various velocities in a closed circuit, and noted the time required to reduce the quantity of CO_2 contained in the air from a certain specified amount to a lower specified amount. He obtained the results given in Table III.

TABLE III.

Wind velocity (metres per min.).	Time (min.).
2.0	73
6.5	57
16.0	39
26.0	28
38.0	22.5
56.0	20

In Table IV I have treated these results in the manner described for the results given in Table II.

TABLE IV.

Wind velocity (m. per min.).	Time (min.).	Time \times (wind velocity) ^{0.39} .
2.0	73	956
6.5	57	1183
16.3	39	1149
26.0	28	998
38.0	22.5	929
56.0	20	961

The products $\text{time} \times (\text{velocity})^{0.39}$ for the different air velocities show good agreement, with the exception of those for velocities 6.5 and 16.0 metres per minute, these are rather higher than the rest. This is also the case in his experiments on photosynthesis, referred to below, where the same velocities are concerned.

In 1933 Heinicke and Hoffman (5) determined the rate of absorption of CO_2 from air, moving with various velocities, by solutions of caustic alkalis of different concentrations. The results are given in Table V.

The products, $(\text{air velocity})^{0.39} \times \text{time}$ required to absorb a specified amount (100 mg.) of CO_2 , have been calculated and are given in Table VI.

These figures provide further strong evidence that the rate of absorption of CO_2 from air by solutions of caustic alkalis is proportional to the air velocity to the power 0.39.

TABLE V.

	Air velocity (l. hr. cm. ²)	CO ₂ absorbed (mg. hr. 100 cm. ²)	Per cent. of CO ₂ removed from air.
A. 0.2 N. KOH. 0.672 mg. CO ₂ per litre of air.	0.42	14.9	53
	0.87	19.8	34
	1.75	24.4	21
	'Free'	25.0	—
	2.63	26.8	15
	5.20	28.1	8
B. N. KOH. 0.544 mg. CO ₂ per litre of air.	0.85	26.9	58
	1.20	33.8	50
	1.90	39.8	38
	'Free'	42.4	—
	2.64	43.1	30
	3.64	44.2	22
C. 0.15 N. NaOH. 0.561 mg. CO ₂ per litre of air.	4.80	46.2	17
	0.25	9.4	67
	0.40	11.0	49
	0.80	13.1	29
	1.83	19.2	19
	2.69	21.7	14
	'Free'	22.7	—
	3.80	23.0	10.8
	4.03	23.4	10.4
	4.47	24.4	9.8

TABLE VI.

	Air velocity l. hr. 10 cm. ²	Time (mins.) to absorb 100 mg. per 100 cm. ²	Time × (air velocity) ^{0.39}
A.	4.2	403	705
	8.7	303	706
	17.5	246	750
	'Free'	240	—
	26.3	224	802
	52.0	214	1000
B.	8.5	223	513
	12.0	178	470
	19.0	151	476
	'Free'	142	—
	26.4	139	498
	36.4	136	552
C.	48.0	130	589
	2.5	638	912
	4.0	546	940
	8.0	458	1031
	18.3	312	970
	26.9	277	1000
	'Free'	264	—
	38.0	261	1077
	40.3	257	1087
	44.7	246	1085

(2) *Photosynthesis.*

Brown and Escombe (1) give no quantitative results with regard to the effect of air velocity on the rate of photosynthesis.

Deneke (4) made experiments on the effect of air velocity upon photosynthesis, using the leaves of several different kinds of plants for the purpose. The air was circulated in a closed circuit, as in the case of his experiments on absorption of CO_2 by caustic alkali solution, referred to above. He quotes numerical results which he obtained with the lower surface of *Ficus elastica* and also with *Tradescantia pendula*; the results with leaves of other plants are shown graphically in his paper, they are, however, similar to those found with the two plants named above. Deneke's results and the calculations which I have made from them are given below in Tables VII and VIII.

TABLE VII.

Lower Epidermis of Ficus elastica.

Wind velocity (m. per min.).	Time (min.).	Time \times (wind velocity) ^{0.39} .
2.0	109	142.8
6.5	83	172.2
16.0	56	165.0
26.0	38	135.4
38.0	29	119.8
56.0	23	110.5

TABLE VIII.

Tradescantia pendula.

Wind velocity (m. per min.).	Time (min.).	Time \times (wind velocity) ^{0.39} .
2.0	148	193.9
6.5	119	247.0
16.0	83	244.6
26.0	61	217.2
38.0	45	185.8
56.0	33	158.5

The products time \times (velocity)^{0.39} for the velocities 6.5 and 16.0 metres per minute are, as mentioned previously, somewhat higher than the others, but on the whole the results indicate that the rate of assimilation by green leaves is proportional to the 0.39 power of the air velocity.

Deneke states that both in the case of alkali solution and leaves 'the absorption of CO_2 reaches a maximum at a velocity of about 100 metres per minute, and cannot be appreciably raised by higher velocities'. It might, therefore, be expected that the product time \times velocity^{0.39} would

tend to become greater at the higher velocities, but this is not the case in the results quoted; the highest velocity included in the tables is, however, only 56 metres per minute.

The results of experiments on photosynthesis made by Heinicke and Hoffman (5) are given in Table IX and my calculations from these results in Table X.

TABLE IX.

No.	Leaf area (cm. ²).	Air supply (l. hr. cm. ²).	CO ₂ assimilated (mg. hr. 100 cm. ²).	Per cent. of CO ₂ removed from air.
1	16.6	7.5	30.8	7
2	20.0	2.5	22.1	14
3	75.5	2.0	21.6	19
4	75.5	1.0	14.5	24
5	204.7	0.6	7.5	32
6	356.6	0.3	5.8	43
7	511.1	0.2	4.7	56
8	1050.0	0.1	2.3	60
9	3625.0	0.024	0.65	77
10	3625.0	0.008	0.36	76
11	3625.0	0.006	0.28	77

TABLE X.

No.	Air supply (l. hr. 100 cm. ²).	Time (min.) to assimilate 0.1 gm. CO ₂ per 100 cm. ²	Time × (air supply) ^{0.88}
1	750.0	195	2576
2	250.0	272	2339
3	200.0	278	2199
4	100.0	414	2497
5	60.0	800	3952
6	30.0	1035	3900
7	20.0	1277	4112
8	10.0	2610	6420
9	2.4	9230	13010
10	0.8	16660	15340
11	0.6	21420	17560

The authors state, with reference to Table IX (their Table IV), 'Nos. 1 to 4 are comparable individual leaves and the values given represent the maximum rate shown on clear days. The data for Nos. 5 to 11 are the results obtained with many leaves enclosed in large cellophane envelopes'— 'the envelopes used with Nos. 9 to 11 measured about 60 × 100 cm.².'

The authors conclude that 'Even though the various determinations are not strictly comparable, since they were not made at the same time, it is clearly indicated that the assimilation rate falls off rapidly if the air supply is less than 2 litres per hour per square cm.'. 'It is evident that up

to a certain limit the total quantity of CO_2 absorbed increases with the supply of air to the chamber, or the rate at which it passes over the KOH . The increases are much more marked at the lower levels of air supply than at the higher levels. These results are in keeping with the findings of Brown and Escombe and of Deneke.'

Here again, however, the more comparable experiments—Nos. 1 to 4—show that the rate of assimilation is proportional to the 0.39 power of the air velocity; the other results—Nos. 5 to 11—also strongly indicate that this may be the case. It is possible that with the lower velocities, from 10.0 to 0.6 litres of air per hour per 100 square cm. of leaf surface, some other factor, e.g. CO_2 deficiency, limited the process.

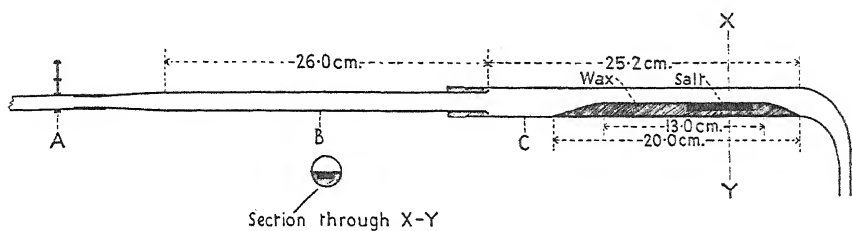
SOLUTION OF SALTS IN WATER.

The correspondence of the effect of air velocity upon the rate of drying hops with its effect upon the rate of absorption of CO_2 from air suggested that a similar relationship might exist between the velocity of a flowing liquid and the rate at which it dissolves a solid over which it flows, this process being fundamentally of a similar nature. To test this hypothesis I have made some preliminary experiments upon the rate of solution of salts in flowing water; these are described below.

In order that the area of the surface of salt exposed to the solvent action of the water should remain constant throughout the experiment, rectangular blocks of the salt were prepared and embedded in paraffin wax, so that the upper surface of the block was flush with that of the wax. Thus only one face of the block came in contact with the water, and the area of this surface was unchanged as the salt passed into solution. As the salt dissolved its exposed surface sank below the level of the wax surface; this was, therefore, scraped down to the level of the salt from time to time; it appears, however, that depression of the surface of the salt block down to approximately 0.5 mm. below that of the wax has no appreciable influence on the rate of its solution.

The apparatus shown in the figure was used for the experiments. Water was allowed to flow by gravity from a constant level tank through the apparatus, its velocity being altered as required by means of the screw clamp, A. The glass tube, B, was 26 cm. long with an internal diameter 1.3 cm. The tube, C, in which the wax block was placed, measured 25.2 cm. from the inlet to the commencement of the curved portion and had an internal diameter of 2.05 cm. A tube of approximately this diameter was chosen so that it would be so large that the alteration in the free path of the water, due to solution of the salt, would make very little difference to the linear velocities corresponding with the volume velocities, also the water in contact with the surface of the salt would be beyond the

influence of the walls of the tube; at the same time the tube would be small enough to bring a considerable fraction of the water into contact with the salt and thus produce a solution of sufficient concentration for easy estimation of the amount of salt dissolved. The wax block was



bevelled at its ends to prevent local stagnation of the water; it was approximately semicircular in section, to fit the tube. The tube was supported horizontally and the surface of the wax block was level; in this way convection currents, due to the difference in density between the solution and the water, were avoided.

The experiments were carried out quickly in order to obviate, as far as possible, change in the level of the surface of the salt; sufficient time was allowed to elapse, however, for the solution to attain equilibrium after each alteration of velocity, before taking a sample for analysis.

The salts used were sodium carbonate, copper sulphate, and rock salt. The block of sodium carbonate was prepared by mixing the anhydrous salt with water to the consistency of a thin paste; this was placed in a mould of suitable dimensions where it solidified to a hard block in a few minutes. After removal from the mould it was trimmed level and rectangular with a file. For the preparation of the copper sulphate blocks large crystals of the salt, clear and free from inclusions, were chosen; blocks were cut from these with a hack saw, and finally smoothed and made rectangular by rubbing on emery paper. The block of rock salt was prepared in a similar manner.

The amounts of sodium carbonate in the solutions were estimated by titration with $N/20$ hydrochloric acid, using methyl orange as indicator; the copper sulphate was determined colorimetrically by means of sodium diethyldithiocarbamate, and the sodium chloride by titration with $N/40$ silver nitrate.

The results of the experiments are given in the following tables, XI-XV.

The experiments recorded in Tables XII and XIII were made with the same block of copper sulphate; the wax block was, however, smaller in the second series, having been scraped down during and after the first series to the level to which the surface of the salt had sunk.

TABLE XI.

Sodium Carbonate. Area of Surface 12.36 sq. cm. Temperature of Water 13.0° C.

No. ¹	Water velocity.		(Velocity) ^{0.39} .	Time (secs.) to dissolve 0.1 gm. Na ₂ CO ₃ , 10 H ₂ O.	Time × (velocity) ^{0.39} .
	Metres per hr.	Litres per hr.			
2	75.8	9.1	2.366	69.8	165.1
5	146.6	17.6	3.061	54.3	166.2
3	220.9	26.5	3.589	50.4	180.8
6	379.1	45.5	4.431	42.6	188.6
4	476.6	57.2	4.848	32.4	157.0
7	675.5	81.1	5.552	28.2	156.5
1	681.5	81.8	5.572	26.5	147.6

TABLE XII.

Copper Sulphate. Area of Surface 5.09 sq. cm. Temperature of Water 13.5° C.

No.	Water velocity.		(Velocity) ^{0.39} .	Time (secs.) to dissolve 0.1 gm. CuSO ₄ , 5 H ₂ O.	Time × (velocity) ^{0.39} .
	Metres per hr.	Litres per hr.			
6	83.4	13.8	2.787	307.4	856
1	240.8	40.0	4.212	204.2	860
3	262.4	43.6	4.357	203.9	888
4	289.0	48.0	4.529	198.4	898
7	322.8	53.6	4.721	169.8	802
5	513.4	85.3	5.662	106.7	604
2	747.0	124.1	6.554	94.9	622

TABLE XIII.

Copper Sulphate. Area of Surface 5.09 sq. cm. Temperature of Water 14.75° C.

No.	Water velocity.		(Velocity) ^{0.39} .	Time (secs.) to dissolve 0.1 gm. CuSO ₄ , 5 H ₂ O.	Time × (velocity) ^{0.39} .
	Metres per hr.	Litres per hr.			
3	166.6	33.0	3.908	209.1	817
6	183.8	36.4	4.060	220.0	893
4	285.3	56.5	4.822	122.2	589
7	430.9	85.3	5.662	114.4	648
1	492.5	97.3	5.957	102.8	612
2	710.0	140.6	6.871	81.3	558
5	727.4	144.0	6.950	84.2	585

The results are in good agreement with the hypothesis that the rate of solution of a substance is proportional to the 0.39 power of the velocity of a solvent flowing over its surface.

The experiments made with copper sulphate show that the ratio of the rate of solution to the water velocity suffers a distinct increase

¹ The numbers indicate the order in which the measurements were made.

when a certain velocity is exceeded; the rates of dissolving below and above this velocity are, in both cases, proportional to the 0.39 power of the velocity, but the proportionality constant is different. In the third series of experiments with copper sulphate (Table XIV) there is also an indication that a second change of a similar nature had taken place before the highest velocity—836.5 metres per hour—was reached. It seems reasonable to suppose that these changes may be associated with changes in the type of flow of the water, e.g. from streamline to turbulent, but this point has not yet been investigated.

TABLE XIV.

Copper Sulphate. Area of Surface 3.20 sq. cm. Temperature of Water 13.75° C.

No.	Water velocity.		(Velocity) ^{0.39} .	Time (secs.) to dissolve 0.1 gm. CuSO ₄ · 5 H ₂ O.	Time × (velocity) ^{0.39} .
	Metres per hr.	Litres per hr.			
7	107.8	16.5	2.985	465.9	1390
5	131.3	20.1	3.221	414.4	1334
3	135.8	20.8	3.266	400.5	1308
1	203.0	31.1	3.819	307.3	1173
4	469.5	71.9	5.297	222.7	1179
6	600.0	91.6	5.824	198.6	1157
2	836.5	128.1	6.637	142.0	943

TABLE XV.

Rock Salt. Area of Surface 6.76 sq. cm. Temperature of Water 15° C.

No.	Water velocity.		(Velocity) ^{0.39} .	Time (secs.) to dissolve 0.1 gm. NaCl.	Time × (velocity) ^{0.39} .
	Metres per hr.	Litres per hr.			
1	200.8	28.5	3.693	58.0	214.0
3	320.4	45.5	4.431	49.3	218.4
6	411.1	58.4	4.881	46.8	228.8
5	537.2	76.3	5.420	38.4	208.0
7	636.5	90.4	5.788	31.6	182.9
2	763.2	108.4	6.223	28.0	174.2
4	1088.0	154.5	7.137	20.7	147.6

Assuming that the effect of an increase in fluid velocity is to reduce the thickness of the stagnant, or slowly moving, layer of fluid in contact with the solid surface, and as the rate of diffusion is inversely proportional to the length of the path of diffusion, it would appear that the thickness of this layer is inversely proportional to the 0.39 power of the fluid velocity.

SUMMARY.

It is shown that the same relationship holds for the effect of the rate of air movement upon the rate of drying hops, the rate of absorption of

CO₂ from air by solutions of caustic alkalis, the rate of absorption of CO₂ by leaves during photosynthesis, and also for the effect of the rate of water flow upon the rate of solution of sodium carbonate, copper sulphate, and rock salt.

The rate in each case is proportional to the 0.39 power of the velocity of the air or water, and, consequently, it appears that the thickness of the 'stagnant film' of fluid is inversely proportional to this power of the fluid velocity.

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The Old Terminology and the New Analysis of Chromosome Behaviour.

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With one Figure in the Text.

THE study of the internal structure of the chromosomes has been carried on for fifty years side by side with the study of their external relationships. Its published results are almost as extensive. But whereas a considerable degree of unanimity prevails with regard to the external relationships, the diversity of opinion with regard to internal structure increases continually. Nor is this due to the inherent properties of the material. Most workers would maintain, properly as I believe, that the chromosomes are much more uniform in their internal than in their external characteristics. The diversity is observational. It is due to the small size of the structures studied and to their great variability under treatment. The problem of internal structure is therefore to a great extent a problem of interpretation, that is, of inference.

In the principles of interpretation I differ from most of my predecessors and contemporaries, though not, I believe, from one whose judgement in this matter I value most highly, Belar. In the study of the chromosomes I attach greater importance to an inference from the comparison of a number of observations than to the direct evidence of a single observation. I prefer the hypothesis to the 'fact'. An observation of fixed material I regard, not as an inescapable fact of life, but as an experiment with life from which one may draw various conclusions depending on its agreement with other similar experiments.

Every observation must be tested by comparison with similar observations. Not only this; each conclusion must be related with every other relevant conclusion. The whole of our information must be integrated into one system. And this system is a constantly increasing one so far as chromosome studies are concerned. If we accept the chromosome theory, genetics and cytology have become one system. We have always had to consider the bearings of each observation of the chromosomes on chromosome

behaviour at every stage in the nuclear cycle at mitosis and meiosis. We must now remember that this behaviour includes what is inferred both from cytological and from genetical study.

In this way we have the chance of correcting our observations of minute and unstable bodies by new tests which give them a certainty they could never have had in the past. The larger the synthesis the greater the value of its consistent components. These views, which I have already described in detail elsewhere (4, Appendix I) have led me to doubt the value of the assumptions underlying the traditional terminology, which allows a morphological value to almost anything seen under the microscope. Such assumptions have come to be used by students as the instruments of their unsuspecting analysis. In consequence a system has been built up in which it is not at once easy to tell what depends directly on observation and what on the concealed assumptions. I therefore propose to examine two of the chief problems of chromosome structure, and endeavour to sort out the essential issues in dispute, from this point of view.

(a) *The Theory of the Pre-resting Stage Split.*

All recent observers of the telophase chromosomes have agreed that they divide at this stage or even earlier. The structural interpretations they give in support of this view are various. Robertson (15 *et al.*) represents the halves resulting from division as lying parallel; Sharp (16) represents them as vaguely coiled round one another; Nebel (14) represents them as coiled in a joint spiral which in the course of telophase contracts to give shorter and straighter threads, and divides into four. These observers are unanimous in disagreeing with the opinion I have given (4, 5) that the chromosomes are always single before the resting stage, and indeed after the resting stage in meiosis.

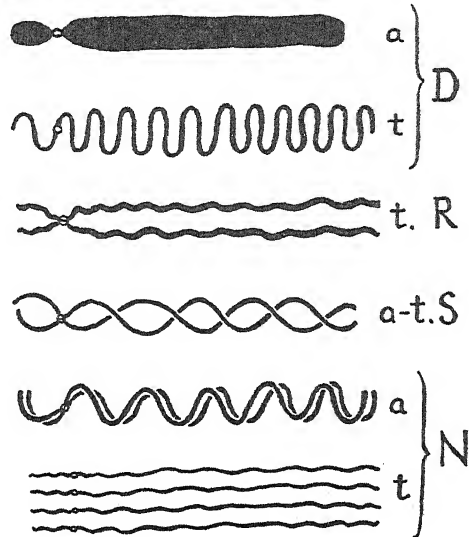
Apart from this negative agreement their interpretations are all incompatible with one another. The split seen by the first observer cuts across the spiral seen by the second; the spiral seen by the second could not be disentangled to give the separation seen by the third. But the observations of a telophase split are not only incompatible with one another; they are incompatible with other available sources of evidence. In the first place, take the evidence of X-ray experiments. About five out of six mutants produced by irradiation of the sperm of *Drosophila* are non-mosaics, and their chromosomes must therefore have been single from the point of view of an X-ray in the resting stage (12). Similarly, mutant nuclei produced by irradiation of root tips in *Crocus* contain only chromosomes with the two chromatids changed at the same place (11). Here again the chromosomes cannot have divided before the end of the resting stage.

What is the cause of this conflict of opinion?

The radius of the coil approaches the wave length of light (5, Fig. 1),

and apparently the special dimensions of the chromatid can be seen to affect the type of optical image, because the appearance or non-appearance of the split depends on (i) the diameter of the chromatid and (ii) the wav-

MITOSIS



MEIOSIS

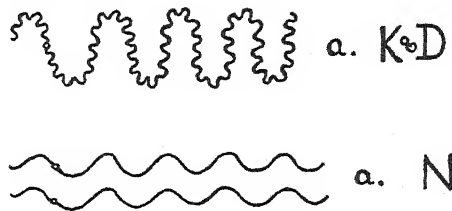


Diagram to illustrate conflicting interpretations of the structure of a chromatid at anaphase (*a*) and telophase (*t*) of mitosis and the first meiotic division. *D*, after Darlington; *K*, after Kuwada; *R*, after Robertson; *S*, after Sharp. Line shows chromosome-thread, gene-string or 'chromonema'; circle shows spindle attachment chromomeres, whose behaviour is unspecified by other authors. The coiling relationship of the two threads is also unspecified by Sharp.

length of light used. Hruby's photographs (6, Fig. 4) show a split with infra-red light where none is visible with ordinary light and where none, I believe, is present.

The issue is clear. Those who believe that the metaphase and anaphase chromatids are split, rest their conclusion on the evidence of effects produced by the agency of radiations of 5,000 Å. This conclusion is reinforced when radiation of 10,000 to 20,000 Å is used. I, who believe that these chromatids are not split, rest my conclusions on different

observations of the same kind, which are reinforced when radiations of 5 to 10 Å are used on living cells. The structures in question probably vary in diameter from 500 to 5,000 Å. It seems that we should have to admit the greater validity of observations made with the finer instrument, even though they were to dispute the ancient authority of our eyes.

But in this case they do not. The evidence of doubleness before the resting stage has necessarily been derived from interpretations of spiralized chromosomes. In these the minor spiral—and even the major spiral—has passed unrecognized. The interpretations have necessarily been variable and incompatible. On the other hand, I find the telophase chromosomes to be single spiral threads (5). The unspiralized chromosomes of the leptotene stage are unanimously admitted to be single, and must therefore have been single before the resting stage. The evidence of X-ray effects agrees with these observations in showing that the chromosomes must be single during the resting stage. The theory that the chromosomes divide before the resting stage is therefore untenable.

(b) *The Theory of the Reticulum and the Matrix.*

The resting nucleus and the metaphase chromosomes are optically homogeneous systems, and we study their internal structure after treating them with heat and with various reagents. In either case the stable system is differentiated into two phases, which differ in refractive index and staining capacity.

Two questions then arise: first, to what extent is this differentiation already present in the living nucleus and in the living chromosome, and secondly, if it is not present, to what extent do the structures revealed by treatment of the resting nucleus and metaphase chromosomes correspond with structures that may be supposed to exist in the living cell?

In considering these questions we must bear in mind that protoplasm, like other protein-water systems, has the property of separating into parts which differ in water content and other associated properties under the action of external or internal stimuli; the natural differentiation in the prophase nucleus is evidently a reversible change of this kind, as shown by the effect on the chromosomes and nuclear sap of dehydrating fixatives. The chromosomes contain less water. When the process is imitated artificially the kind of differentiation arising depends on the kind of stimulus used, just as when the curd separates from the whey in milk its water content depends on the stimulus effecting the separation. This enables us to understand why the staining part of the chromosome may be uncontracted by fixation in some cases, where no external structure is revealed, while in others it is slightly contracted (5, Fig. 4), or even very heavily contracted (5, Fig. 3). These differences in the artificial product make it unnecessary for us to suppose that any such differentiation exists in the living

material; it is an artifact. But the structure it reveals may correspond in certain relationships, such as the internal spiral arrangement, with the structure of the living chromosome; it may be a *characteristic artifact* (4, App. 1).

This must be the case when the artificial differentiation given by mechanical or osmotic stimuli is, like the natural one, reversible (3).

The test of whether the structure revealed by the artifact is present in the living cell must therefore be a test of the results of different treatments at one stage and of similar treatments at different stages. These show that the thread-bundle structure of the resting nucleus inferred by Belar from the appearance of living cells (3 *et al.*), and shown by aceto-carmine fixation, agrees with that produced artificially at metaphase by the technique of Kuwada and Nakamura (8), and also with the expectation from the uncoiling of the chromosome threads that I have observed at telophase (5). It is therefore a characteristic artifact and a sound basis of interpretation. On the other hand, the reticulum produced by other fixations (cf. 16) varies according to the technique used, as Belar has pointed out (1, pp. 5 and 11), and cannot be reconciled with any structural changes in the nucleus that can be observed or inferred. It has the morphological validity of the cracks in dried cheese. It is a non-characteristic artifact, and useless for any purpose beyond 'cell-corpse histology' (*Zelleichen-histologie*).

The separation of the chromosomes into stainable and non-stainable parts, a curd and a whey, which reveals the valid spiral structure and the invalid reticulum, has been made the basis of a distinction between a staining 'chromonema' and a non-staining 'matrix' lying around it. This is a morphological distinction. There are several grounds—chiefly non-morphological—for assuming that the chromosome thread has some sort of pellicle (4, 5), but there are no grounds for equating the various separations of weak-staining parts of chromosomes effected by fixation with any such organ. As Belar has stated (1, p. 15): 'Chromatin ist also ein weder färberisch noch morphologisch, sondern ein morphogenetisch charakterisierter Begriff.' That is, the test of the validity of its interpretation depends on developmental comparison. There are no grounds for supposing that the differentiation between 'chromonema' and 'matrix' is a characteristic differentiation of substances unless these can be shown to have developmental continuity.

Let us see how these expressions are used by an authority who is widely followed. Sharp describes the matrix in these terms (16, p. 106): 'This is a substance of uncertain history' and 'it is probable that in some form and amount it accompanies the more stainable chromonema at all times'. During prophase we learn of 'the translucent matrix', and since the nuclear sap is also translucent the matrix is presumably invisible. But 'at this

period the matrix develops a strong affinity for the stains commonly employed, so that the whole chromosome may look like a solid body with no internal structure'. At anaphase, however, separation of the more watery constituents is at last possible, and we are told therefore (p. 135) that 'each chromosome consists of a matrix and chromonema' but 'the chromaticity of the matrix usually obscures this structure unless special methods are employed'—unless, that is, the chromosome thread is contracted by special fixatives. 'As the telophase begins, the chromaticity of the matrix decreases markedly, so that the chromonemata are more easily observed'—in other words, the minor spirals relax. And then we come to the reticulum: 'the chromonemata of the several chromosomes become joined together by anastomoses to form the reticulum. The origin and nature of these connecting strands is uncertain', except in *Zea*, where it has fortunately been possible to show, according to Sharp, that 'they are clearly formed by the matrix'. However, 'little is known about the fate of the matrix in the telophase' apart from (p. 136) 'the possibility that in nuclei generally the matrix may maintain its identity from telophase to prophase in the form of anastomoses, nucleolar matter, and possibly thin sheaths about the chromonemata'.

The evidence of a pellicle or of other accessory materials in the chromosomes is functional. The use of 'matrix', on the other hand, rests on morphological evidence. It is the word for a 'substance'. From the quotations given the history and dimensions, structure and function of the *substance* may appear uncertain. The properties of the *word*, however, are straightforward and clear. Its origin is in observation, the observation of gaps appearing in stained chromosomes with convenient fixation. Its function is to fill the gaps in our knowledge of the chromosomes. First it filled the morphological gaps. Now it is beginning to fill the physiological ones. Soon, when they are noticed, it will be used to fill the genetical and mechanical gaps.¹

The term 'matrix' gives a unity of terminology to a multiplicity of events. It gives a continuity of description to a discontinuity of behaviour. It conceals and maintains a hundred conjectures for the use of those who would never wittingly make one hypothesis. And consequently it enables an apparently logical and unshakable structure to be erected on premisses which its authors have long forgotten. The 'matrix' has become a myth.

I take these examples not merely to discuss the particular issues involved, although they are important, but in order to show what the essential conflict of method in cytology is about. It does not depend merely on details of observation or individual prejudices. It is a conflict

¹ Metz (*Proc. Nat. Acad. Sci.*, xx, 159, 1934) has promised to do this.

between two habits of thought. The older habit depends on applying the same principles to cytology as to histology, and with equal certainty. It gives the same faith to the interpretation of structures of small size as of large size. It attaches the same value to observations of fixed protoplasm as of fixed cellulose. It consequently finds the co-ordination of extensive series of observations unnecessary, and the intervention of genetics irrelevant except in formal discussion. Facts are facts and have to be accepted, however improbable. It finds its unity and consistency in developing etiological myths on the basis of a learned terminology, such as 'matrix', 'chromonema', 'cytomixis', 'genome', 'perigenesis', 'heterotypic mitosis', 'catenation' and a dozen other such words with implications that are vitalistic, teleological, or entirely uncertain. Behind this verbal screen a body of esoteric literature has developed, so well protected from understanding that the most critical observers on the outside have often been fain to dismiss the whole study as an imaginative imposture. They have been able to show on physico-chemical grounds how naïve many cytological interpretations have been, and they have thrown out the good with the bad and indifferent.

This is no longer necessary. We are now in a position to build up a new system of chromosome study. It must find its unity and consistency in rigorously applying working hypotheses, founded on data derived from every available method of study, particular and statistical, mechanical and physiological, experimental and comparative. In doing so it has to discard the myth-terminology that has grown up on the forgotten basis of undefined assumptions. The new method cannot work with the old words. It has to describe its subject-matter in terms fitted for their uses; if hypotheses are to be rigorously applied their terms must be rigorously defined. The new system will then be harder to understand than the old one. But we shall know when we do understand it, and it will therefore be easier to criticize.

We have reached a new epoch in the study of chromosomes when we want to know, and are able to find out, not merely what they look like when they are dead, but what they do when they are alive.

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An Anatomical Study of the Leaves of the Carboniferous Arborescent Lycopods.

BY

ROY GRAHAM, Ph.D.

With fifty-three Figures in the Text.

THE investigation of the structure of the foliar organs of fossil plants is a neglected field. Various leaves have been described from time to time, but, with the exception of Thomas's (31) work on the leaves of *Calamites*, there have been no systematic studies of the anatomy of the foliage of a particular genus or family. The leaves of the carboniferous arborescent lycopods (*Lepidodendraceae*) are considered in this paper.

The following is a brief historical résumé of anatomical studies of leaves belonging to this group. In 1879 Renault (21) described and figured the leaves of *Sigillaria* sp. and *Sigillariopsis Decaisnei* Ren. (This latter is probably not a *Sigillaria*, see below.) In another paper (22) he described and figured the leaves of *Lepidodendron esnotense* Ren. and of *Sigillaria Brardi* Br. with its varieties *spinulosa* and *latifolia*. Scott (23) described *Sigillariopsis sulcata* Scott and (24) figured that species, and also figured a leaf which he found associated with *Lepidodendron Hickii* Wats. Arber and Thomas (1) also figured *Sigillariopsis sulcata*. Seward (26) gave outline drawings of isolated leaves belonging to either *Lepidodendron* or *Sigillaria*. Stopes (30) twice figured the same transverse section of the apical region of a *Lepidostrobus* cut above the axis, the section passing through the upturned sporophyll laminae. One figure is described correctly, the other is described as a section through a bud of *Lepidodendron* showing many small leaves packed around the axis. Recently Koopmans (18) described another species, *Sigillariopsis laevis* Koop. Calder (3) recorded poorly preserved leaves under the name *Sigillariophyllum elegans* Calder.

In the present study the writer has had to deal largely with isolated sections of leaves. In no case did he have serial sections, and in only two cases were the leaves attached to twigs, thus permitting determination of the plane of section, and only in one case was correlation with the stem species possible. It has been found convenient to group the leaves into types as Type A, B, &c. Owing to differences in the shape and structure

of basal and apical sections of the leaves of some species (cf. Figs. 29 and 30 and Figs. 52 and 53) it is possible that in dealing with isolated sections that a single species of leaf may be included in more than one type. Conversely it is possible that the leaves of two species may be so similar that they may be included in the same type. Only the more sharply defined types, founded on fairly well preserved material, have been named.

Except where the leaves are found in organic connexion with twigs, it is impossible to correlate the leaves with the various species or even genera of stems. The features enumerated by Renault (22) to distinguish *Lepidodendron* and *Sigillaria* leaves cannot be used with any great degree of certainty. The writer has therefore placed isolated leaves with a single vascular strand in the genus *Lepidophyllum* without necessarily implying that they belong to the stem-genus *Lepidodendron* rather than to *Sigillaria* or one of the other stem-genera. Leaves with a double xylem strand are placed in the leaf-genus *Sigillariopsis*. These leaves probably belong to *Sigillaria*, since in the Carboniferous *Lepidodendraceae* a double xylem trace has been found only in certain species of *Sigillaria*.

The wide range of structure in the leaves of the *Lepidodendraceae* is far greater than that to be found within two of three genera of a comparable group of plants such as the *Coniferales* and *Cycadales*. This fact suggests that the leaves of a number of diverse genera of the *Lepidodendraceae* may have been confused. That the group is very diverse is also shown by the wide range in the structure of the fructifications (cf. *Lepidostrobus*, *Spencerites*, *Bothrodendron*, *Mazocarpon*, and *Lepidocarpon*), differences which, if they occurred in living plants, would be used to separate families or subfamilies.

The leaves of the *Lepidodendraceae* arise in close spiral sequence, and in the case of *Sigillaria* appear to form numerous vertical rows. The leaves are usually long and narrow, but show considerable diversity in external form. 'In some species, as for example in *Lepidodendron longifolium* Br., they have the form of long and acicular needles very similar to those of *Pinus longifolium*; in *L. Sternbergii* they are much broader and shorter' (26) and resemble in general shape the leaves of *Taxus baccata* L. In size the leaves range from a few millimetres in length, as in *L. veltheimianum* Sternb. and *L. selaginoides* Sternb., to a length exceeding 15 cm. in *Lepidodendron obovatum* (34), or even attaining a length of a metre, as in *Sigillaria lepidodendrifolium* (26). Some species of leaves have the stomatal areas sunken below the general leaf surface, thus forming two stomatal furrows on the abaxial surface of the leaf.

The structure of the persistent leaf-base is well known, and has been adequately treated in the many papers dealing with the anatomy of the stem. The parichnos was dealt with in considerable detail by Hill (15) and Weiss (33). The presence of a ligule, borne in a pit on the upper

surface of the persistent leaf-base, was demonstrated almost simultaneously by Solms-Laubach (27) in *Lepidodendron veltheimianum* Sternb. and by Hovelacque (14) in *L. selaginoides* Sternb. It has subsequently been demonstrated in a number of other species. Arber and Thomas (1) describe the ligule in *Sigillaria scutellata* Br. Kidston (17) did not mention the ligule in an otherwise detailed description of *Sigillaria elegans* Br. A well-preserved petrified stem of *Sigillaria approximata* Font. and White, examined by the writer, revealed no trace of a ligule. It is possible that in these cases the ligule was borne on the leaf itself, as in most species of *Selaginella*, and was therefore shed with the leaf (32).

The anatomy of the actual leaf-blade will now be considered.

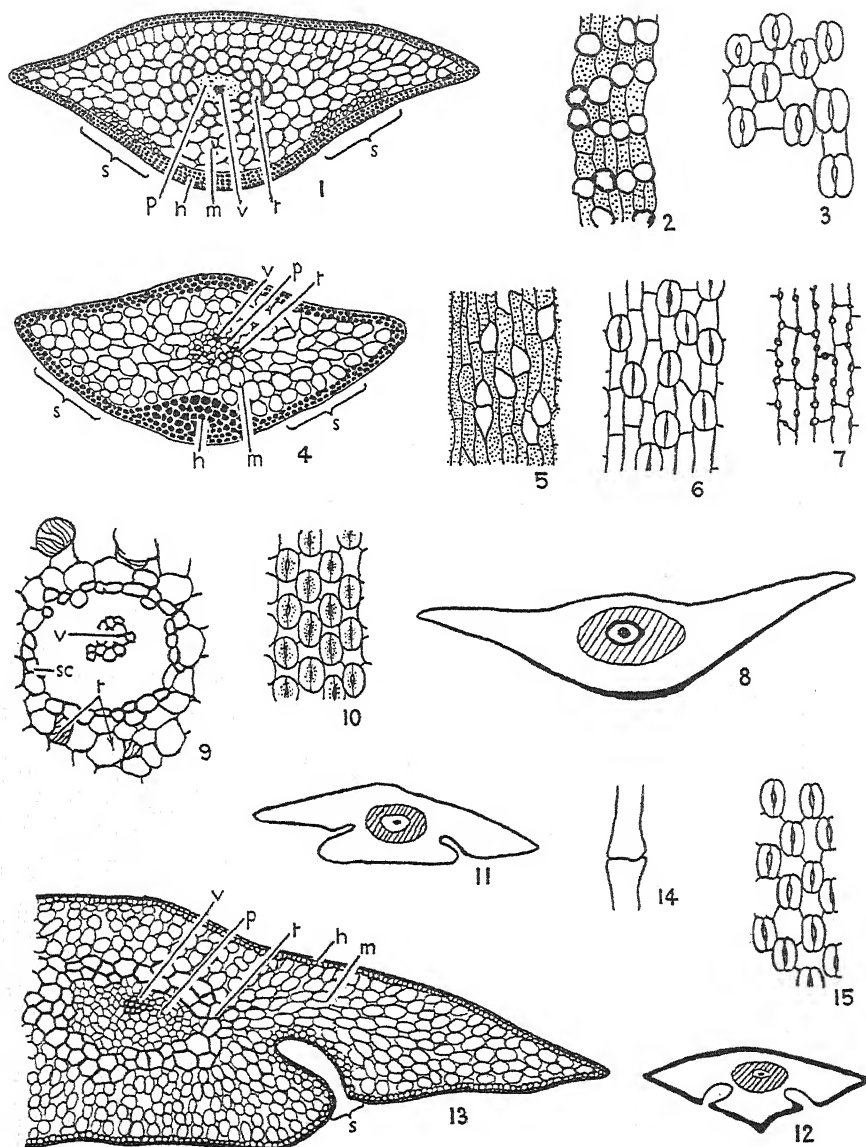
Xylem.

The xylem occurs as a single strand except in *Sigillariopsis*, where it is double (Figs. 48 and 51). The amount of xylem varies in different species from half a dozen elements, as in Type B (Fig. 4), to the large bundle of Type H 1 (Fig. 32) which consists of three or four dozen tracheides. This variation in the amount of xylem is in part correlated with the size of the leaf. The elements may occur in a circular group, as in Types A, B, and D (Figs. 1, 4, and 13), or the bundle may be more or less transversely elongated, as in Types H and I (Figs. 32, 40, and 42). In *Sigillaria Brardi* (Figs. 52 and 53) the transverse elongation of the bundle is extreme, the bundle consisting of a band of tracheides a single cell in width. In the species with very small bundles it has not always been possible to determine the position of the protoxylem. In the large bundles of Types H 1, H 3, and I (Figs. 32, 40, and 42) no spiral elements were observed, but the smaller elements occupy the ends of the transversely elongated bundle, and presumably this represents the position of the protoxylem. In *Sigillariopsis sulcata* and *S. halifaxensis* the smaller elements occupy the distal ends of each of the xylem bundles. Renault (22) found that the protoxylem of *Lepidodendron esnotense* is at the ends and along the abaxial margin of the bundle. In *Sigillaria Brardi* it is at the ends of the bundle. The metaxylem in all the species consists of scalariform tracheides. There is no secondary xylem.

Phloem.

For purposes of description only, the tissues surrounding the xylem and occupying the region between the xylem and transfusion tissue are termed phloem.

In Type H 1 (Fig. 32) the phloem is developed in greater amount on the abaxial side of the xylem. Two distinct types of tissue are present, a thin-walled parenchyma *p* immediately surrounding the xylem, and two groups of thick-walled cells *sc* below the bundle. The position of these



FIGS. 1-15.

FIGS. 1-3. Type A (Leaves of *Lepidodendron veltheimianum*). Fig. 1. Transverse section (K 85). $\times 35$. Figs. 2 and 3. Surface sections of epidermis (W 47) and stomata (K 88). Both $\times 170$.

thick-walled cells in relation to the bundle is similar to that of the thick-walled sieve-tubes of *Isoetes Hystrix* and *I. velata* (19, 25). The parenchyma cells are elongate with transverse end walls. Some are peculiar in having expanded ends (Fig. 35). It is not known whether this is the real shape of the cells or merely an artifact caused by shrinkage of the cells prior to fossilization. The general aspect of the tissue is that of phloem, though no sieve areas could be recognized in the fossil material. The cells of the sclerenchyma strands are elongate and have transverse end walls (Fig. 36). Their shape is similar to the thick-walled sieve-tubes of *Isoetes*, though here again no sieve plates could be recognized.

In all the other species the xylem is in the same way surrounded by thin-walled parenchyma; the sclerenchyma, when present, varies in amount and distribution from species to species. It may occur as a continuous arc, as in Type L (Fig. 51), or in two distinct groups, as in Type K (Fig. 48). In Type M (Figs. 52 and 53) the thick-walled elements form a triangle enclosing thin-walled tissue. In some species, as Type L (Fig. 51), the sclerenchyma strands curve around the ends of the xylem bundle so as to partially surround it. In Type C (Fig. 9) the sclerenchyma forms a uniform sheath completely surrounding the xylem and parenchyma.

In a number of species the phloem contains no sclerenchymatous elements. In these the parenchyma is usually developed equally on all sides of the xylem (Fig. 1), but in some it is more abundant on the abaxial side (Fig. 13). In Type D this tissue was studied in longitudinal section. It consists of thin-walled cells with transverse end walls (Fig. 14). They are similar to the thin-walled phloem elements of Type H 1 (cf. Fig. 35).

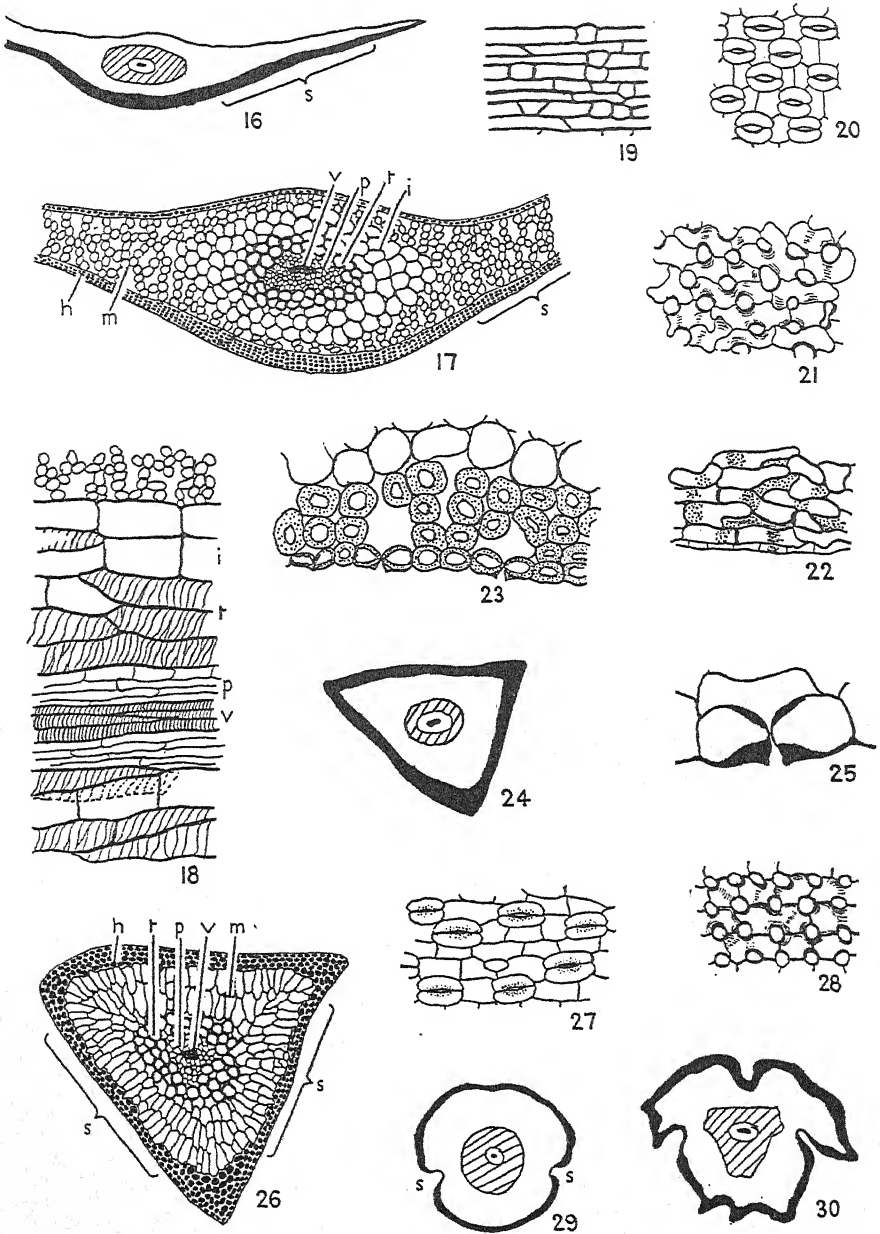
Transfusion tissue.

In most of the species studied this tissue completely surrounds the xylem and phloem, forming a sheath from two to four cells in width. In the two species of *Sigillariopsis* studied, and in *Sigillaria Brardi* the transfusion tissue is small in amount and occurs only as a discontinuous arc on the abaxial side of the bundle. According to Renault's figures (22) the transfusion tracheides of *S. Brardi* are arranged in radial rows. The transfusion tracheides are fairly short elements with more or less transverse end walls and coarse reticulate thickenings (Figs. 18 and 33). Their

FIGS. 4-7. Type B (*Lepidophyllum minor*). Fig. 4. Transverse section (B 574). $\times 62$. Figs. 5, 6, and 7. Surface sections of epidermis, stomata, and of sub-stomatal hypoderm (B 574). All $\times 170$.

FIGS. 8-10. Type C. Fig. 8. Diagrammatic transverse section (G 44). $\times 35$. Fig. 9. Detail of bundle (G 46). $\times 170$. Fig. 10. Surface section of stomata (G 44). $\times 170$.

FIGS. 11-15. Type D (*Lepidophyllum Sewardi*). Fig. 11. Diagrammatic transverse section (S 51). $\times 30$. Fig. 12. Diagrammatic transverse section (S 1967). $\times 14$. Fig. 13. Transverse section (S 51). $\times 85$. Fig. 14. Sieve-tube? (S 51). $\times 250$. Fig. 15. Surface section of stomata (S 51). $\times 170$.



FIGS. 16-30.

FIGS. 16-23. Type E (*Lepidophyllum latifolium*). Fig. 16. Diagrammatic transverse section (Q a 1). $\times 12$. Fig. 17. Transverse section (Q a 2). $\times 62$. Fig. 18. Longitudinal section (Q a 1). $\times 170$. Figs. 19 and 20. Surface sections of epidermis (Q a 11) and of stomata (Q a 7). Both $\times 170$. Figs. 21 and 22. Surface and longitudinal section of sub-stomatal hypoderm (Q a 1). $\times 170$. Fig. 23. Transverse section of stomata and sub-stomatal hypoderm (Q a 1). $\times 330$.

diameter is usually much greater than that of the true tracheides. Exterior to, and sometimes intermingled with the transfusion tracheides, are cells of a similar cylindrical shape, but with unthickened walls (*z*, Figs. 18 and 33). Their function is probably also one of conduction.

The mesophyll.

The mesophyll of the various species exhibits a considerable range of structure. In some (Fig. 4) it consists of more or less isodiametric cells loosely packed together, in others (Fig. 40) it is a very lacunar tissue composed of chains of cells, and, in still others (Fig. 26) palisade tissue is very well developed.

Hypoderm.

This tissue consists of thick-walled elements which may well have provided strength to the leaf. The amount of this tissue varies from species to species, but there is some correlation with the size of the leaf. In the very small leaved species, as Type A (Fig. 1), or Type D (Fig. 13), the hypoderm is small in amount. In some of the larger species of leaves as Types H (Fig. 32) and J (Fig. 45), there is a great amount of this tissue.

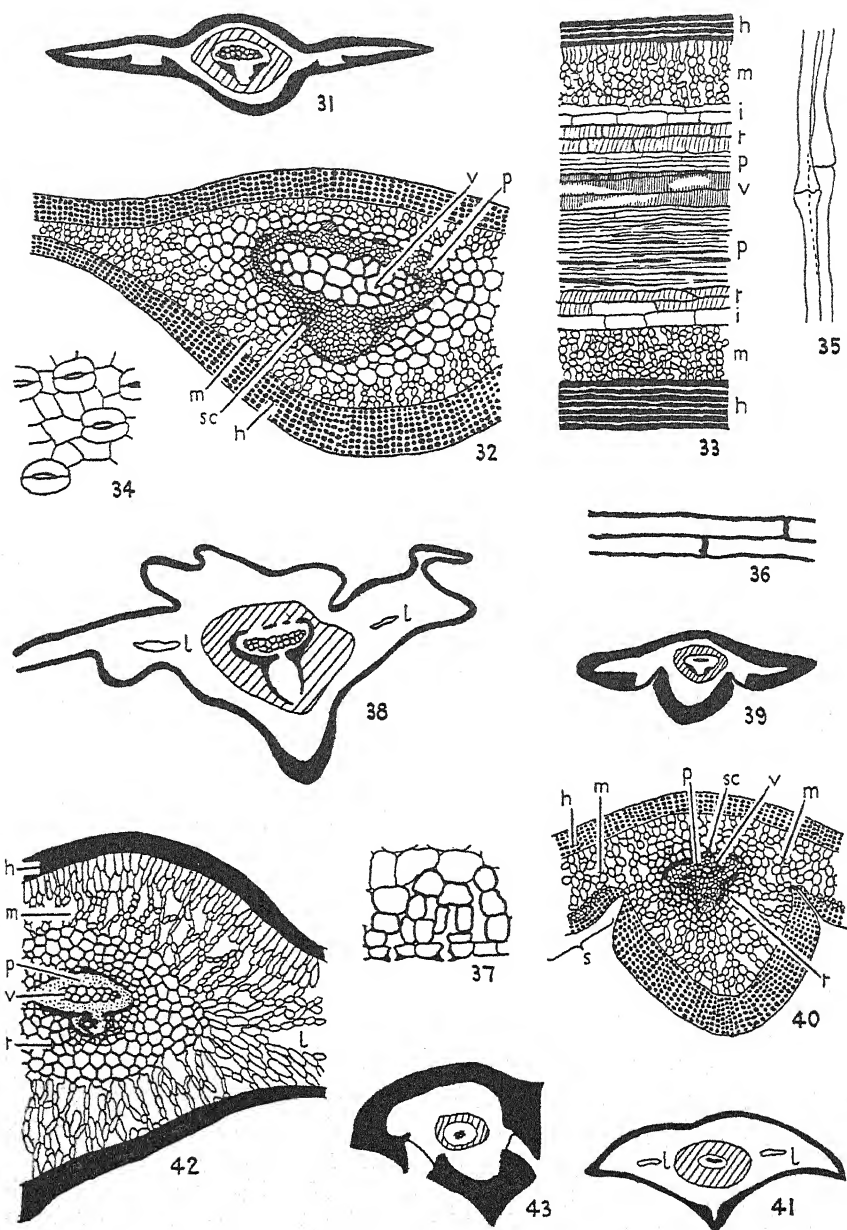
Except immediately beneath the stomatal areas the hypoderm consists of a continuous layer of very elongate thick-walled cells with sharply tapering ends. Beneath the stomatal bands the hypoderm is either absent or is modified to permit the passage of air. In general it is absent from the stomatal areas of those species with stomatal furrows. The modified hypoderm of the stomatal bands may consist of more or less rectangular cells fitting closely together, but with small air passages maintained (Fig. 7). In other cases the cells are irregularly shaped and interlocking (Figs. 21 and 22). The thick-walled hypoderm cells thus constitute an aerenchyma, which, from its structure, appears to have had considerable mechanical strength. Figs. 23, 28, and 37 also illustrate the structure of this tissue. The writer has not met a similar type of tissue in any other group of plants. The nearest analogy is found in *Xanthorrhoea*, and in many Restionaceae, where the respiratory cavity is bounded by thick-walled cutinized cells (9, 20).

Epidermis and stomata.

The stomata in all the species studied are limited to two bands, one each side the mid-vein on the abaxial surface of the leaf. In some species

FIGS. 24-8. Type F (*Lepidophyllum equilaterale*). Fig. 24. Diagrammatic transverse section (Q a 1). $\times 46$. Fig. 25. Transverse section of stoma (Q a 1). $\times 700$. Fig. 26. Transverse section of leaf (Q a 1). $\times 62$. Figs. 27 and 28. Surface sections of stomata and of sub-stomatal hypoderm (Q a 8). $\times 170$.

FIGS. 29 and 30. Type G (Leaves of *Lepidodendron esnotense*). Diagrammatic transverse sections (after Renault's figures). Fig. 29 near apex, and Fig. 30 near base of leaf. $\times 15$.



FIGS. 31-43.

FIGS. 31-37. Type H 1 (*Lepidophyllum Thomasi*). Fig. 31. Diagrammatic transverse section (B 587). $\times 12$. Fig. 32. Transverse section (T 2). $\times 38$. Fig. 33. Longitudinal section (T 2). $\times 38$. Fig. 34. Surface section of stomata (S 2597). $\times 170$. Fig. 35. Thin-walled cells of 'phloem' (S 2597). $\times 105$. Fig. 36. Thick-walled cells of 'phloem' (T 2). $\times 105$. Fig. 37. Transverse section of stomata and underlying hypodermis. (S 2483). $\times 185$.

the stomatal areas are sunken beneath the general surface of the leaf in well-marked stomatal furrows.

There is a great difference in the structure of the epidermis of the non-stomatal and the stomatal areas. In the former the epidermis consists of two kinds of cells: elongate, more or less rectangular cells, interspersed with much shorter rounder cells, which appear to be of lighter colour in surface section. There is considerable variation in the shape and relative number of the two kinds of cells (cf. Figs. 2, 5, and 19). In all the species examined the cell-walls are non-sinuous.

The stomata are closely crowded. Subsidiary cells are few, one cell frequently abutting on the guard cells of two or more stomata. From the relations of the end walls of the guard cells to the subsidiary cells, the latter appear to have originated from a different mother-cell from the former—Florin's (11) 'Ursprungliche' (original) stomatal type. There is but a single cycle of subsidiary cells. The stomata are all orientated parallel to the length of the leaf, and are level with the epidermis. There is considerable variation in the degree of crowding of the stomata and in the number, shape, and size of the subsidiary cells (cf. Figs. 3, 6, 10, 20, 27, and 34).

In section the guard cells have the same structure as that of some modern Pteridophytes, including *Lycopodium* (4). The ventral half of both the inner and the outer walls is thickened, and the outer wall frequently bears a projecting ridge (Figs. 23, 25, 37, and 49). In connexion with the guard cells the writer uses the term 'ventral' for that part of the guard cell adjacent to its neighbour: 'inner' and 'outer' refer to the surface turned toward the leaf mesophyll or exposed to the surface, respectively.

Descriptions of the various species studied follow. The letters used in designating the slides studied are as follows: B, Botany School, Cambridge; G, the writer's own collection; K, Geology Department, King's College, London; Q a, Binney Collection, Sedgwick Museum, Cambridge; T, collection of Dr. H. H. Thomas; S, Scott Collection, British Museum; W, Williamson Collection, British Museum.

The illustrations require a word of explanation. The cross sections of the leaves are given by diagram and restorations. In the diagrams the outline of the leaf and of the regions of the principal tissues are drawn by camera lucida, the shading is entirely diagrammatic. In the restorations the outline of the leaf and of the tissue regions are drawn by camera

FIG. 38. Type H 2. Diagrammatic transverse section (B 575). $\times 14$.

FIGS. 39 and 40. Type H 3 (*Lepidophyllum papillonaceum*). Fig. 39. Diagrammatic transverse section (B 575). $\times 14$. Fig. 40. Transverse section of same. $\times 37$.

FIGS. 41 and 42. Type I. Fig. 41. Diagrammatic transverse section (S 2011). $\times 7$. Fig. 42. Transverse section of same. $\times 21$.

FIG. 43. Type J (*Lepidophyllum angulatum*). Diagrammatic transverse section (S 2084). $\times 14$.

lucida, and the cells are sketched in to scale, but without any attempt to draw in each individual cell as it appears. There is some restoration, especially of the softer tissues. Figures of the epidermis, stomata, and hypoderm are actual camera lucida drawings of the cells.

Type A. (Leaf of Lepidodendron veltheimianum Sternb.)

K 85, 177, 1302, 1303, W 471.

Locality: Pettycur, Fife. Horizon: Lower Carboniferous.

Figs. 1 to 3.

Leaves 8 to 11 mm. in length by 1.5 to 2 mm. in width. Leaf triangular in section, wider than thick. Upper surface of leaf flat or slightly convex. The lower surface of the leaf is much more convex. There are no stomatal grooves. The xylem consists of a circular group of from six to twelve spiral and scalariform tracheides, but the position of the protoxylem was not determined. The xylem is surrounded by thin-walled tissue, usually badly preserved. Surrounding this is a zone of transfusion tracheides about two cells in width. The mesophyll consists of loosely packed cells, which are slightly elongated in the direction of the axis. The hypoderm is from two to four cells in thickness, and is somewhat thicker in the lower face of the leaf. It is continuous beneath the stomatal bands. The epidermis consists of rectangular and circular cells in approximately equal numbers. They are arranged in longitudinal rows. The circular cells are paler in colour than the other cells, and some contain dark incrustated material (Fig. 2). The stomata are separated by an approximately equal number of subsidiary cells. The guard cells are 30μ long by 10 to 12μ wide. The subsidiary cells measure 15 to 25μ .

The leaves of this species were found attached to small twigs. The section just described was cut near the base. Near the apex, the leaf section is somewhat narrower, but otherwise it has the same form and structure.

Type B. (Lepidophyllum minor sp. nov.)

B 574, 575, 588.

Locality: Shore, Littleborough. Horizon: Lower Coal Measures.

Figs. 4 to 7.

Leaves about 6 mm. long and 1 mm. wide. Leaf lenticular in cross section, about twice as wide as thick, the lower side being the most curved. There are no stomatal furrows. The xylem consists of a very small group of three to six spiral tracheides. The details of the delicate tissue immediately surrounding the xylem could not be made out. Below the bundle about half a dozen transfusion tracheides occur in a discontinuous arc, the individual tracheides sometimes being separated by parenchyma cells. In

the leaf-base the transfusion tissue is much more abundant and surrounds the xylem. The mesophyll consists of loosely packed, more or less isodiametric parenchyma cells. The hypoderm is strongly developed on the mid-line of the abaxial face of the leaf, where it is from six to eight cells in width. In other parts of the leaf it is only about two cells in thickness. It is continuous beneath the stomatal areas. The epidermis of the non-stomatal areas consists of greatly elongated, more or less rectangular cells. The guard cells of the stomata are 25 to $30\ \mu$ long. The number of subsidiary cells is greater than the number of stomata. The subsidiary cells are rectangular in shape, and are elongated in the direction of the leaf, and are 20 to $40\ \mu$ long by about $15\ \mu$ wide. The sub-stomatal hypoderm consists of rectangular cells with numerous small air passages between the cells.

The leaves of this species are attached to small twigs, but the twigs are so small, and their steles not sufficiently well preserved to permit of identification. There is but little variation between the various leaves of this species, and the cross-section of the leaf changes but little from base to apex.

The leaf is named *Lepidophyllum minor* on account of its small size.

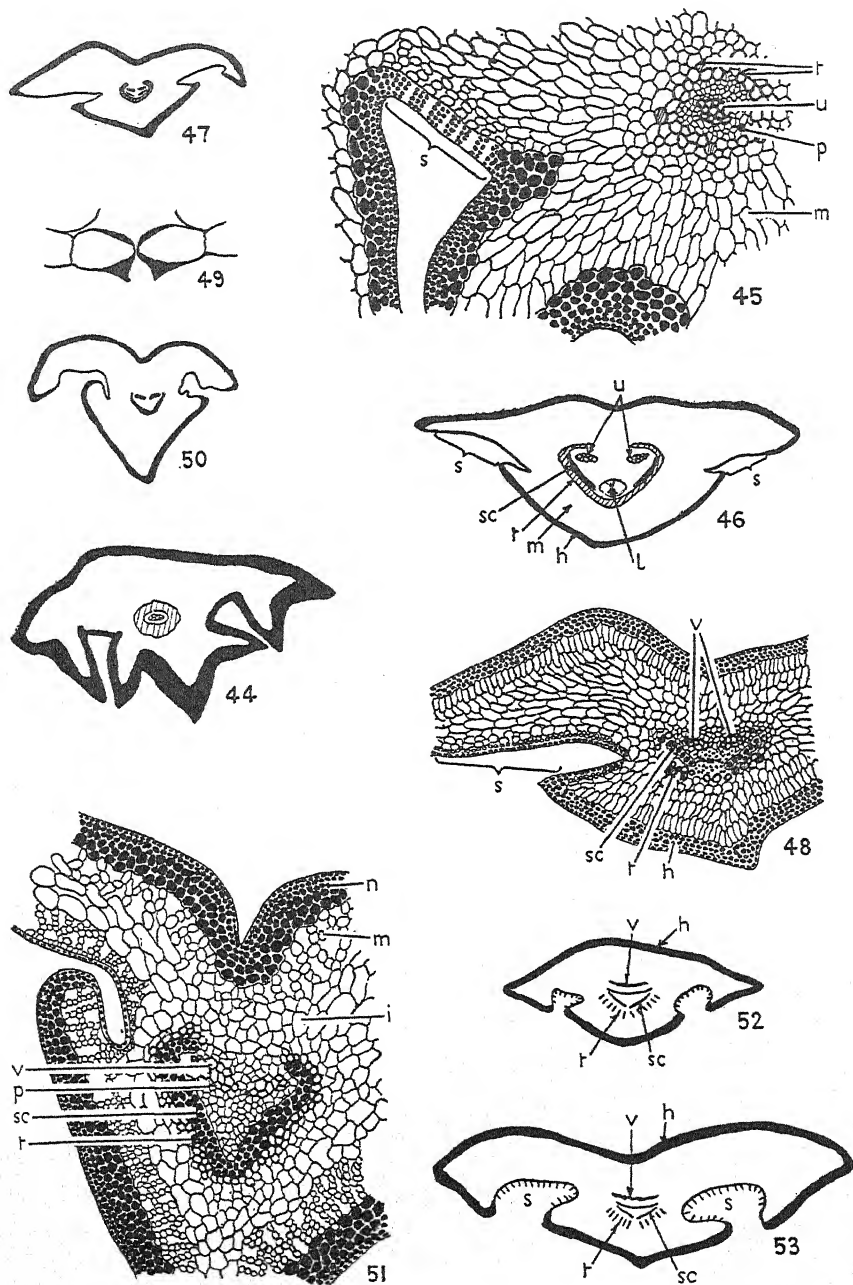
Type C.

G 44, 46.

Locality: Calhoun, Illinois. Horizon: Middle Conemaugh (Upper Coal Measures).

Figs. 8 to 10.

Many poorly preserved isolated leaves are found in association with twigs which resemble *Lepidodendron selaginoides* Sternb. The leaves are 1.5 to 2.0 mm. in width, and are three to five times as wide as thick. The upper surface is more or less flat; the lower, convex. There are no stomatal furrows. The xylem strand is circular, and consists of about a dozen small spiral and scalariform tracheides. Surrounding the xylem is a space formed by the disintegration of delicate tissue. Outside this is a circular sheath of thick-walled elements (*sc*, Fig. 9). Surrounding this is a variable amount of transfusion tissue. The mesophyll is completely disintegrated in most of the specimens, but in a few there are the remains of chains of small cells. In the upper surface of the leaf the hypoderm is absent or consists of a single layer of cells. In the lower surface of the leaf it is two or three cells in thickness. The stomata are very crowded, and occur in longitudinal rows. The guard cells are $25\ \mu$ in length. Alternating with each pair of guard cells is a single subsidiary cell about $10\ \mu$ in length. This species has the most closely spaced stomata of any of the species examined.



FIGS. 44-53.

FIGS. 44 and 45. Type J (*Lepidophyllum angulatum*). Fig. 44. Diagrammatic transverse section (S 94). $\times 14$. Fig. 45. Transverse section (S 94). $\times 60$.

FIGS. 46-49. Type K (*Sigillariopsis sulcata*). Fig. 46. Diagrammatic transverse section.

Type D (Lepidophyllum Sewardi sp. nov.).

S 51, 1867.

Locality: doubtful. Horizon: Lower Coal Measures.

Figs. 11 to 15.

The type specimen is S 51, which forms the basis of the description. The leaf is of unknown length, is 1.2 mm. in width and 0.2 mm. in thickness. The leaf is roughly lenticular in shape. The upper surface is convex, the lower surface much more so. The narrow U-shaped stomatal grooves about 0.15 mm. in depth occur in the lower surface of the leaf. There is no ridge at the mid-line of the lower surface of the leaf.

The xylem bundle is circular in cross-section, and consists of about a dozen tracheides. It is surrounded by thin-walled tissue, which is more abundant on the lower side of the xylem. In longitudinal sections of other leaves in the same slide this tissue is seen to consist of elongated thin-walled cells with transverse end walls (Fig. 14). The ends of the cells are expanded, but this appearance may be an artifact produced by shrinkage of the cell. Although no sieve-plates could be recognized, the writer believes that these cells may be sieve-tubes. This tissue is surrounded by a zone of transfusion tissue about two cells in width. The mesophyll consists of loosely packed, slightly elongated cells which radiate from the bundle. The hypoderm consists of a single cell layer, which is absent from the stomatal bands. The stomata occur on both surfaces of the stomatal groove. A single subsidiary cell alternates regularly with each pair of guard cells in longitudinally running rows. The guard cells measure 27 by 9 μ . The subsidiary cells are 18 to 30 μ in length.

Another specimen is illustrated in Fig. 12. The leaf is slightly larger and relatively thicker than the type specimen. It measures 2.2 mm. wide by 0.8 mm. thick. Unlike the type specimen, it has a sharp ridge at the mid-line of the lower surface. The hypoderm is from two to three cells in thickness. This specimen differs slightly from the type specimen, and may, or may not, belong to the same species.

Scott (25) figures the type specimen as the leaf of *Lepidodendron Hickii* Wats. In the type specimen these leaves are found along with another species of lepidodendroid leaf associated with a stem of *Lepidodendron Hickii*. In the writer's opinion the leaves probably did not belong to the stem, and the correlation with the stem species is not warranted. This species is named after Professor A. C. Seward.

(S 2272). $\times 13$. Fig. 47. Diagrammatic transverse section (B 586). $\times 13$. Fig. 48. Transverse section (B 586). $\times 34$. Fig. 49. Transverse section of stroma (B 586). $\times 350$.

FIGS. 50 and 51. Type L (*Sigillariopsis halifaxensis*). Fig. 50. Diagrammatic transverse section (S 1901). $\times 13$. Fig. 51. Transverse section of same. $\times 60$.

FIGS. 52 and 53. Type M (Leaves of *Sigillaria Brardi*). Diagrammatic transverse sections after figures by Renault. Fig. 52, near base, and Fig. 53, near apex. $\times 10$.

Type E (Lepidophyllum latifolium sp. nov.).

Q a 1, 2, 8, 11.

Locality: doubtful. Horizon: Lower Coal Measures.

Figs. 16 to 23.

There are a number of isolated sections which show a considerable range in size. The length of the leaves is unknown. They range in width from 2 to 6 mm., and are usually more than six times as wide as thick. The upper surface of the leaf is flat or sometimes slightly concave; the lower is convex. There are no stomatal furrows.

The xylem consists of about a dozen tracheides, and is slightly elongated in the tangential direction. It is surrounded first by a zone of thin-walled elements which are greatly elongated parallel to the bundle, and then by a zone of transfusion tissue about two cells in width. The transfusion tracheides are much wider than the xylem tracheides, and have coarse reticulate thickenings (Fig. 18). Outside the transfusion tracheides is a zone of large cells which are elongated parallel to the axis (*i*, Figs. 17 and 18). These cells have transverse end walls. They are wider and shorter than the transfusion tracheides, and have no thickenings on their walls. Their function was probably one of conduction.

The mesophyll is very lacunar, and consists of chains of small cells. The hypoderm is absent, or only one cell in thickness, at the upper face of the leaf, but is from three to six cells in thickness at the lower. Figs. 21 to 23 show the structure of the sub-stomatal hypoderm, which consists of irregularly shaped thick-walled cells with large air passages between. The stomata are closely spaced. They are ranged in longitudinal rows, with one or two subsidiary cells between each pair of guard cells. The guard cells are short and wide, measuring $26\ \mu$ by 10 to $12\ \mu$.

The section in Q a 2 may be considered the type specimen. The name of the species refers to the broad leaf-section.

Type F (Lepidophyllum equilaterale sp. nov.).

Q a 1, 2, 3, 8.

Locality: doubtful. Horizon: Lower Coal Measures.

Figs. 24 to 28.

There are about half a dozen transverse sections and several oblique and longitudinal sections. The species exhibits very little variation. The leaves are of unknown length, and are from 0.6 to 1 mm. in width. The leaf-section is an equilateral triangle, the upper surface of the leaf forming one side, and the lower surface the other two sides. The stomata occur in the lower two sides of the triangle. There are no stomatal grooves.

The circular xylem strand consists of about a dozen tracheides, and it is surrounded by a narrow zone of thin-walled tissue, and then by transfusion tissue two or three cells in width. The entire mesophyll consists of palisade tissue, the palisade layer being about three cells in depth. The hypoderm is two to four cells in thickness, and is thicker at the angles of the leaf. It is continuous beneath the stomatal areas. The stomata are not so closely spaced as in the other species examined; there are three or four subsidiary cells to every pair of guard cells. The guard cells are large, about 35μ in length. The subsidiary cells are more or less rectangular, and measure 20 to 40μ long by 15 to 20μ wide.

This species derives its name from the shape of the cross-section of the leaf.

Type G (Leaves of Lepidodendron esnotense Ren).

Locality: Autun, France.

Figs. 29 and 30.

See Renault (22) for detailed figures and descriptions. The leaves are 1.5 to 2.0 mm. wide at the base, and are several cm. long. Note the difference in shape between the apical section (Fig. 29) and basal section (Fig. 30). The mesophyll consists almost entirely of palisade tissue.

In its internal structure this leaf is very similar to Type F, but is easily distinguished from that species by the entirely different shape of the cross-section.

Type H 1 (Lepidophyllum Thomasi sp. nov.).

S 2580, 2581, 2597, T 2, B 587.

Locality: Shore, Littleborough. Horizon: Lower Coal Measures.

Figs. 31 to 37.

The type specimen, upon which the description is based, is in slide T 2. The leaves are of unknown length and are up to 6 mm. in width. The leaf has a large prominent midrib 1 mm. thick. The wings of the lamina are about a third of the thickness. There are no stomatal furrows. The midrib is more prominent on the lower than on the upper surface of the leaf.

The xylem strand is very large. It is transversely elongated and measures 0.6 by 0.2 mm. It consists of large scalariform tracheides. No spiral elements were observed, but the smaller tracheides occupy the ends of the bundle, and these probably indicate the position of the protoxylem points.

The xylem is surrounded by a thin-walled tissue which has a much greater development on the lower side of the bundle. The cells of this region are elongated. They have expanded ends and transverse end walls

(Fig. 35). Their general appearance is that of sieve-tubes, though no sieve-plates could be recognized. At the outer margin of this thin-walled tissue are some thick-walled elements. They occur mainly in two groups below the bundle and also extend around the ends of the xylem bundle. A few thick-walled elements are also found above the bundle, so that this tissue forms an incomplete sheath to the bundle. It consists of thick-walled cells with transverse end walls (Fig. 36). Their position in relation to the bundle and their shape recall the thick-walled sieve-tubes of *Isoetes*. No sieve-plates could be seen in the fossil material. It is equally possible that this tissue is merely collenchyma associated with the bundle.

Exterior to the thick-walled cells is a zone of transfusion tracheides one or two cells in width, and then one or more layers of cells similar in shape but without the reticulate thickenings (*z*, Fig. 33). The mesophyll is a very loose tissue composed of chains of small cells. The hypoderm in the non-stomatal areas is from five to ten cells in width. It is thickest immediately below the midrib. It consists of radially arranged rows of cells. In the stomatal areas the hypoderm is three to four cells in thickness. The stomata are not closely crowded, and there are two or three polygonal subsidiary cells for every pair of guard cells. The guard cells are $35\ \mu$ in length.

Other specimens as B 587 deviate from the type only in minor differences in shape.

The species is named after Dr. H. H. Thomas.

Type H 2.

B 574, 575.

Locality: Shore, Littleborough. Horizon: Lower Coal Measures.

Fig. 38.

There are two adjacent sections cut from the same leaf. The leaf is of unknown length. In section it is roughly triangular in shape, and is somewhat distorted due to crushing. It is 4.5 mm. in width and 2 mm. thick. The closely packed isodiametric mesophyll cells, similar to the tissue of a leaf base, and the existence of lacunae (*l*, Fig. 38) in the position of parichnos, suggest that the specimens are basal sections of a leaf. The vascular bundle has the same structure as that of H 1. This leaf is in all probability a basal section of that, or of a closely similar species of leaf.

Type H 3 (Lepidophyllum papillonaceum sp. nov.).

B 574, 575.

Locality: Shore, Littleborough. Horizon: Lower Coal Measures.

Figs. 39 and 40.

The type is founded on two adjacent sections of two leaves. Both are slightly crushed and the mesophyll is in a poor state of preservation.

Leaves of unknown length, 2.5 mm. wide and 1 mm. thick, at the midrib. The thick centre part of the leaf is separated from the lateral wings of the lamina by stomatal furrows 0.2 mm. in depth. The cross-section of the leaf has a shape similar to that of a thick-bodied moth. The xylem strand is tangentially elongated and measures 0.2 by 0.5 mm. The structure of the vascular bundle and hypoderm is similar to that of Type H 1. The fragments of mesophyll still preserved indicate that this tissue was lacunar and consisted of chains of cells.

This species is distinguished from *Lepidophyllum Thomasi* by the narrower, relatively thicker cross-section; the presence of stomatal grooves; and by the relatively smaller vascular bundle.

The specific name refers to the cross-sectional shape.

Type I.

S 2011.

Locality: Moorside, Oldham. Horizon: Lower Coal Measures.

Figs. 41 and 42.

This is a rather poorly preserved leaf of roughly rhomboidal outline. It is 5 mm. wide and 2 mm. thick. There is a shallow groove in its upper surface. Stomatal grooves are absent. The specimen is probably a section cut near the base. The bundle is of the same form and relative size as Type H 3. There is a very great amount of transfusion tissue which forms a zone four to six cells in width. A palisade tissue is present in the upper surface of the leaf. The remainder of the mesophyll is very lacunar and consists of chains of cells. The hypoderm is completely carbonized and shows no structure.

Type F (*Lepidophyllum angulatum* *sp. nov.*).

S 94, 95, 96, 2084.

Locality: Yorkshire. Horizon: Lower Coal Measures.

Figs. 43 to 45.

S 95 is the type specimen upon which the description is based (Figs. 44 and 45). Leaf of unknown length, 3 mm. broad, and 1.5 mm. thick. On the abaxial side of the leaf are two stomatal grooves with flat bottoms. These grooves divide the leaf section into three lobes: a central and two lateral. The widest part of the grooves is at the bottom. They measure 0.5 mm. in depth and are 0.25 mm. wide at the bottom. In the lower surface of the leaf there are also two shallow V-shaped grooves in the central lobe and one in each of the lateral lobes. Although crushing has to some extent accentuated these grooves, they are not entirely caused by crushing.

The vascular bundle is small in comparison with the cross-section of the leaf. It consists of a circular xylem strand of about a dozen tracheides, surrounded by a narrow zone of thin-walled elements and then by a zone

of transfusion tissue two cells in thickness. The mesophyll consists of slightly elongated cells which radiate from the bundle. The hypoderm is five to eight cells in thickness. The inner layers of the hypoderm consist of cells of much larger diameter than those of the outer layers. A modified hypoderm is present beneath the stomatal areas. A badly crushed, but similar leaf also occurs in the same preparation as the type.

In another slide, S 2084, there is a leaf differing in shape (Fig. 43) which may or may not belong to the same species as the type. The leaf is 2 mm. wide and 1.5 mm. thick. The leaf is triangular, and there are two large stomatal furrows with the stomata limited to the flat bottom of the groove. Unlike the type specimen there are no other grooves in the surface of the leaf. The hypoderm is absent from the stomatal areas. The structure of the various tissues is similar to that of the type specimen.

The species derives its name from the very sharply angular outline of the leaf section.

Type K (Sigillariopsis sulcata Scott).

S 2268, 2272, B 586.

Locality: Shore, Littleborough. Horizon: Lower Coal Measures.

Figs. 46 to 49.

See Scott (23 and 24) for the original description and figure. Also Arber and Thomas (1) for another illustration. Fig. 46 is from Scott's type specimen S 2272. The leaf is 3.5 mm. wide and 1.55 mm. thick. It is roughly triangular in shape. The stomatal grooves are narrowly V-shaped. The xylem occurs as a double strand. The protoxylem, marked by the smaller elements, is found at the outer ends of each of the bundles. Two arcs of thick-walled cells occur beneath the bundle. Between these groups is a lacuna (l, Fig. 46) caused by the disintegration of thin-walled tissue. The transfusion tissue forms an arc to the outside of the thick-walled elements. The individual transfusion tracheides are separated one from another by parenchyma cells. A palisade tissue is found on the upper surface and the flanks of the leaf; the remainder of the mesophyll consists of fairly closely packed isodiametric cells.

The specimen B 586 (Figs. 47 and 48) exhibits some differences. The leaf is 2.5 mm. wide, and is wider in proportion to its thickness than the type specimen. The stomatal furrows are wider and deeper. Palisade tissue has a greater development than in the type specimen, the greater part of the mesophyll having a palisade structure. This leaf may or may not belong to the same species as the type specimen.

Type K I (Sigillariopsis laevis Koopmans (18)).

A number of leaves in a not very good state of preservation were named by Koopmans *Sigillariopsis laevis*. Koopmans says 'The only

important differences with *Sigillariopsis sulcata* Scott are the total absence of stomatal furrows and the presence of a distinct keel on the dorsal face of the leaf'.

Type K 2 (*Sigillariophyllum elegans* Calder (3)).

Poorly preserved leaves found in close association with *Sigillaria elegans* and with very similar structure to *Sigillariopsis laevis* Koop. were named *Sigillariophyllum elegans*.

Type L (*Sigillariopsis halifaxensis* sp. nov.).

S 1901.

Locality: Halifax Hard Beds. Horizon: Lower Coal Measures.

Figs. 50 and 51.

This species is founded on a single specimen. The leaf is of unknown length, is 2 mm. wide and 1.3 mm. in thickness. There is a slight median groove in the upper surface and two stomatal grooves in the lower. The grooves are distorted by crushing of the leaf, but they appear to have been U-shaped instead of V-shaped as is the case in *Sigillariopsis sulcata*.

The xylem occurs as a double strand, each strand consisting of about a dozen tracheides. The smaller elements are found at the outer end of each of the bundles. Surrounding the xylem is a zone of thin-walled tissue which is developed mainly on the lower side of the xylem. Below this is a V-shaped band of thick-walled cells. At the outer margin of this thick-walled tissue is a zone of transfusion tissue one or two cells in width. The inner part of the mesophyll consists of closely packed fairly large cells, more or less isodiametric in section. The outer part of the mesophyll consists of a lacunar tissue composed of chains of cells. The hypoderm is five or six cells in thickness; the inner cells are of larger diameter than the outer. There is no hypoderm beneath the stomatal areas.

This species differs from *Sigillariopsis sulcata* in the following respects: the greater depth of the leaf; the greater development of the thick-walled elements associated with the bundle, and which occur as a single band and not as two distinct groups; and in the outer layers of the mesophyll having a chain-like, instead of a palisade structure.

Type M (*Leaves of Sigillaria Brardi* Br.).

Locality: Autun, France.

Figs. 52 and 53.

See Renault (22) for detailed figures and original descriptions of the species and its varieties *spinulosa* and *latifolia*. The leaves are several centimetres in length and are 5 to 6 mm. wide at the base. They taper gradually towards the tip. There are two wide U-shaped stomatal furrows in the lower surface of the leaf.

The xylem strand is greatly elongated tangentially, and is a single cell in width. The protoxylem occur at the ends of the bundle. Below the xylem strand are three bands of thick-walled cells, forming a triangle enclosing softer tissue. Below the sclerenchyma bands are radially arranged groups of transfusion tissue. The mesophyll of the flanks of the leaf consists of transversely elongated cells. The wide stomatal furrows are beset with multicellular epidermal hairs. The tangentially elongated mesophyll occurring just above the large stomatal furrows give the leaf the appearance of having the power of rolling, similar to that of certain grasses.

Sigillariopsis Decaisnei Ren.

See Renault (21 and 22) for detailed descriptions and figures.

The leaf is triangular in cross-section and is without stomatal furrows. In the lower part of the leaf there is a double xylem strand, but the two bundles unite towards the apex of the leaf. The bundle contains secondary as well as primary xylem. Both primary and secondary xylem have pitted elements in addition to the usual scalariform tracheides. Below each of the xylem strands is an arc of thick-walled tissue. Below the whole bundle is a zone of transfusion tracheides. The hypodermal elements are grouped into fascicles. The stem to which the leaves belong also contains pitted tracheides.

This species shows striking peculiarities in the occurrence of pitted tracheides, both in the stem and leaf; in the presence of secondary xylem in the leaf; and in the fascicled hypoderm. These peculiarities which led Renault to consider the species as an intermediate type between *Sigillaria* and *Poroxyton*, lead the writer to the conclusion that the affinities of Renault's species are not with *Sigillaria* but with some gymnosperm allied to the Cordaitales.

A comparison was made between the leaves of the Lepidodendraceae and those of the modern Lycopodiales. In the structure of the stomata the genera *Lycopodium*, *Selaginella*, and *Isoetes* agree with the Lepidodendraceae. There is a single cycle of subsidiary cells which are derived from different mother cells from the guard cells, and the thickenings of the guard-cell walls are similar. Apart from the structure of the stomata, and the fact that there is but a single vascular bundle, there are no further points of resemblance between the leaves of the Lepidodendraceae and those of *Lycopodium* and *Selaginella*. The resemblances to *Isoetes* are confined to the stomata and to the vascular bundle. In all other features the leaves are as different as possible.

All the leaves studied exhibit xeromorphy, but in different degrees. The presence of a thick hypoderm, well-developed transfusion tissue

and stomata sunken in grooves are xeromorphic features. *Sigillaria Brardi* shows additional features in the presence of hairs lining the stomatal grooves and in the leaves probably possessing the ability of rolling. As xeromorphy in plants may occur under many widely different conditions, it would be unwise to draw conclusions as to climate from the structure of the leaves. The similarity in structure to the leaves of the conifers is striking, but it does not seem probable that this resemblance is of any phylogenetic significance.

SUMMARY.

1. A morphological study has been made of the leaves of the carboniferous *Lepidodendraceae* and the literature reviewed.

2. The leaves described vary somewhat from the forms generally given in text-books, especially in the fact that in several species the stomata are not situated in grooves.

3. There does not appear to be any fundamental difference between leaves of *Sigillaria* and those of *Lepidodendron*. All leaves with a double xylem strand are attributed to *Sigillaria*, but this character is not constant for that genus.

4. The vascular bundles show considerable variation, and some of the species have a flattened bundle with two lateral protoxylem groups.

5. In several species thick-walled tissue is associated with the bundle. This is regarded as probably being similar in nature to the phloem fibres and sieve-tubes of *Isoetes*.

6. Transfusion tissue is usually strongly developed.

7. Considerable variation is shown in the form of the photosynthetic tissue.

8. Hypodermal strengthening tissues are always present, and are greatly developed in the broader leaves. The hypodermal tissue beneath the stomatal areas has an interesting structure.

9. The form and structure of the stomata are very uniform in all species examined, and resemble that of modern lycopods.

10. A number of new species are described.

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The Metabolism of Calcareous Algae.

II. The Seasonal Variation in Certain Metabolic Products of *Corallina squamata* Ellis.

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With six Figures in the Text.

IN a previous communication (3) attention was drawn to the constant occurrence of peptides in certain marine Algae, and the hypothesis was reached that their presence is due to a lack of balance between the carbon and the nitrogen metabolism, owing to the depression of carbon assimilation caused by low light intensity or by long periods of aerial exposure. An obvious test of the hypothesis is a periodic crop-analysis of a suitable plant; that is, a plant which contains not only an appreciable quantity of peptide but also of sugar. One such is *Corallina*, since it contains a pentapeptide of aspartic acid, and floridoside, a galactoside of glycerol (3); further, its constant immersion and its thick incrustation of carbonate makes illumination a limiting factor for the greater part of the year. No detailed study of seasonal metabolism in such growth-conditions as *Corallina* experiences appears to have been published; for us the attempt was made possible by the finding of an almost pure stand of *C. squamata* at Dancing Ledge on the Dorset coast, in sufficient quantity for many collections.

An area was carefully selected and prescribed and the gatherings were made throughout the year by the same person. The original proposal was to collect at regular intervals, but this intended regularity was not possible, for the plant, being always submerged on this particular site, can only be collected at low water of spring tides and then only if the sea be reasonably calm: when the sea was rough collection had to be postponed to the next spring tides.

The material was picked over in the laboratory, air dried, and analysed with as little delay as possible. Much preliminary work was done to find

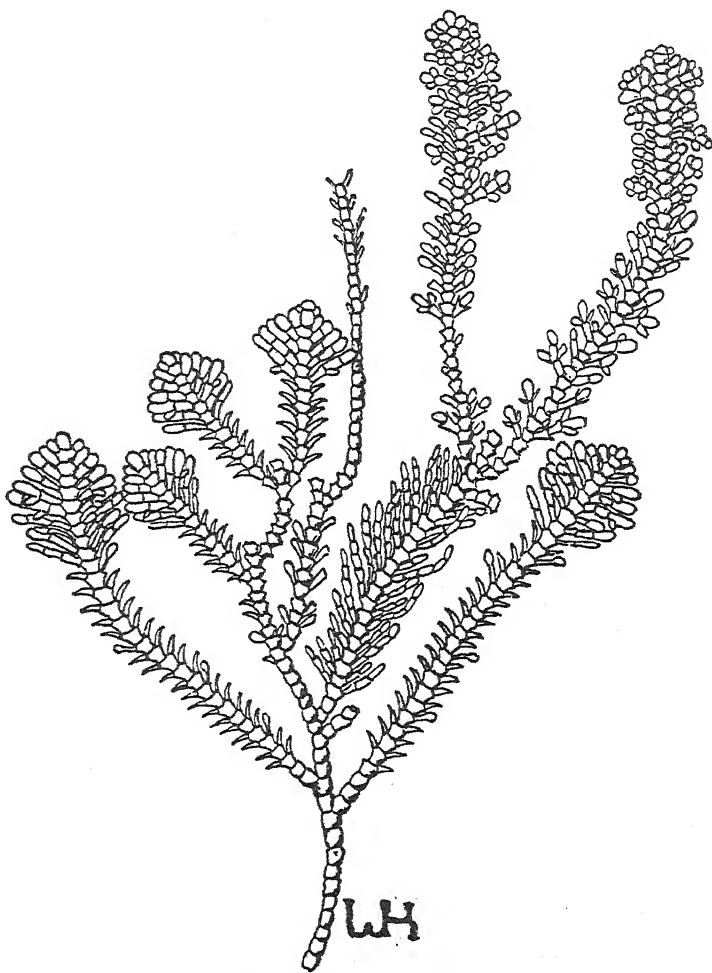


FIG. 1. Material collected on 22 April 1934. $\times 3$.

the most suitable methods of analysis, and to systematize every detail of procedure in order to obtain correct relative values.

MORPHOLOGY.

Before passing on to the main work, some mention must be made of the vegetative development. During the winter months the plant is found in dense tufts about 3 cm. long. In the early spring begins the formation of young shoots from the basal regions and also of lateral branches. Towards the end of May growth becomes more active and the plants are

fully grown in July, the tufts being very dense, about 6 cm. in length, and having the maximum development of lateral branches. In the autumn

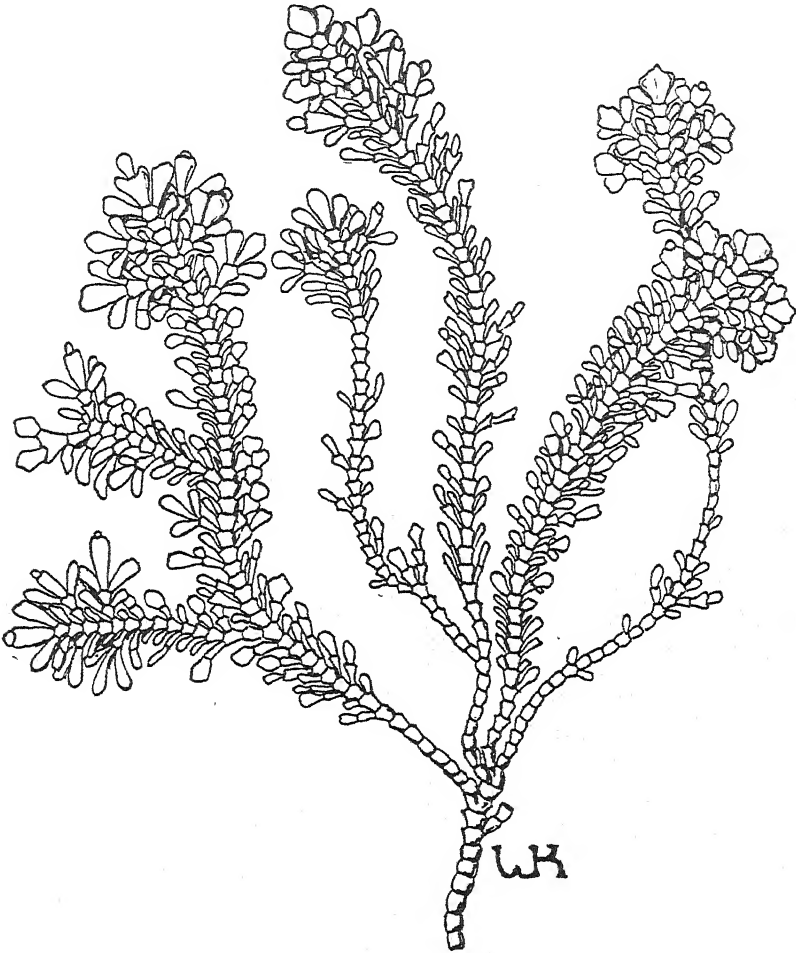


FIG. 2. Material collected on 28 May 1934. $\times 3$.

there is much shedding of the mature shoots, their place being taken by new growths.

Conceptacles are obvious throughout the growing season.

The appearance of typical portions of thallus at different times of the year are shown in Figs. 1-4.

MINERAL INCRUSTATION.

The incrustation of 'calcareous' Algae is, in the main, a mixture of the carbonates of calcium and magnesium; the ratio between the two is

R r

apparently never constant, and the magnesium is always much less than the calcium. The analyses of Clarke and Wheeler (1) have likewise shown the occurrence of magnesium with calcium in calcareous algae and in the exoskeletons of many marine invertebrates. Various suggestions have been

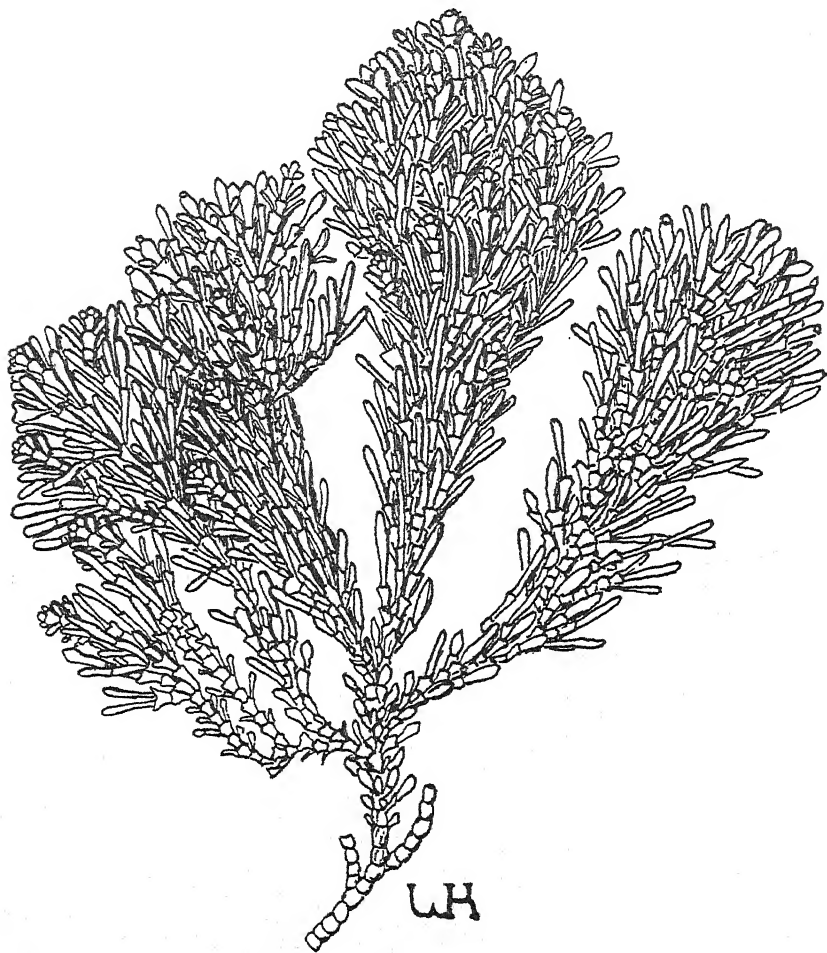


FIG. 3. Material collected on 1 July 1934. $\times 3$.

made to account for the formation of the incrustation, but these cannot be dealt with here; the subject is fully discussed in a paper by Pia (4), in which a comprehensive bibliography will be found.

An aspect of the problem which has not hitherto received attention is the possible variation in the amount of the mineral incrustation at different periods of the year. On investigation it was found that such a variation does occur, and for this reason it became necessary to refer all determinations of the metabolic constituents of the plant to the basis of the oven-

dried weed minus its incrustation. Such a procedure was in effect a compromise, inasmuch as no correction was made for the ash content proper of the weed, as distinct from its external coating of carbonate, but this, in the circumstances, was inevitable. For the purpose of obtaining some

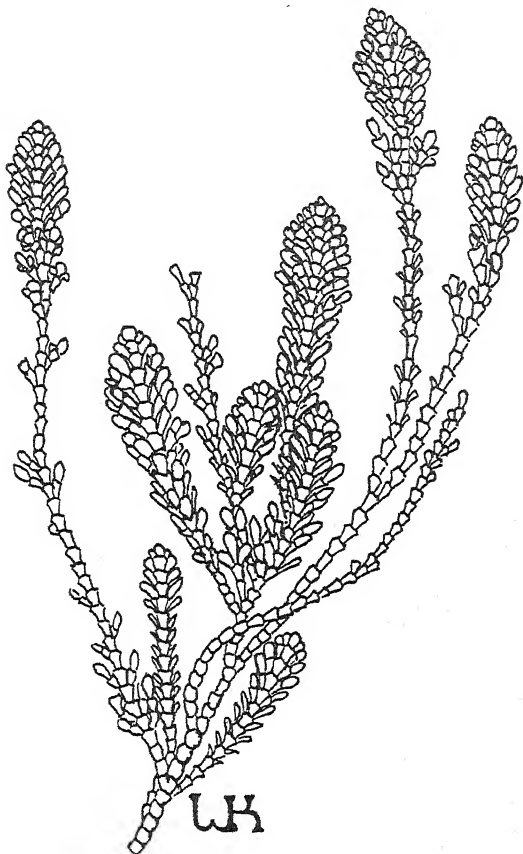


FIG. 4. Material collected on 12 September 1934. $\times 3$.

measure of the amount of the incrustation, a known weight of the weed was dried to constant weight and then treated with an excess of standard hydrochloric acid. When the effervescence had ceased, the excess of acid was titrated back with standard alkali.¹ On calculating the percentage of carbon dioxide in the weed a small yet significant seasonal variation was found to occur. These values, although a true index of the carbonate content, are useless for calculating the absolute amounts of the organic constituents of the plant.

¹ It was found necessary to add a small amount of powdered sugar to the solution before titration in order to dissolve the calcium hydroxide, which was liable to precipitation.

In utilizing the titration figures for calculating the actual weight of incrustation, two methods suggested themselves. The first was to convert the titration figures into their equivalent of calcium carbonate on the ground that, although the incrustation was known to contain some magnesium carbonate, the amount present was not sufficient to make any appreciable difference in the conversion factor, and would at most give a titration figure which was slightly too high. The alternative method of estimating the total calcium and magnesium in the solution after titration, and converting these respectively into their equivalent weights of carbonate, was found to give values (summation figures, $a + b$, Table I) which invariably exceeded the titration figures, which themselves were admittedly high. The results showed that the estimations of total calcium and magnesium in the titrated solution included some of these metals not present in the weed as carbonate. The summation and titration values, calculated on the dry weight of the plant, are given in Table I and plotted in Fig. 5.

In view of these facts, it was decided to adopt the titration figures as a basis for calculation, and hence the weight of true weed minus carbonate.

TABLE I.

	CaCO ₃ (a).	MgCO ₃ (b).	a + b.	Titration figures.
1933				
Nov. 29	71.68	10.53	82.22	81.87
1934				
Jan. 29	72.42	9.65	82.07	81.64
Mar. 26	70.40	9.26	79.66	79.16
May 28	71.05	8.67	79.72	79.32
July 1	72.34	9.54	81.88	81.85
Aug. 13	71.29	9.79	81.08	80.52
Sept. 12	71.37	10.34	81.71	81.46
Oct. 10	72.46	10.19	82.65	82.21

An examination of these curves shows that the magnesium carbonate diminishes from November to March, reaching its minimum towards the end of that month. There is then a gradual increase to September, followed by a slight fall. This regularity is not shown by calcium carbonate, which also is minimal in March, but has two maximal peaks in January and August. The values of the combined carbonates, whether found by summation or by titration, closely correspond and are highest in the winter and lowest from the end of March to the end of May. This is followed by a sharp rise in June, whilst in August there is a fall and then a rise to the winter value. The two depressions in the total carbonate curve may be explained by the shedding of mature branches and the development of new shoots which occurs in the spring and autumn, for analysis has shown that the younger parts of the thallus contain less carbonate (79.49 per cent.) than do the older (80.81 per cent.).

DISTRIBUTION OF NITROGEN.

It was found possible to estimate the total nitrogen of the plant on the samples which had been titrated for carbonate, a considerable advantage, since sampling errors were minimized and economy of plant material and

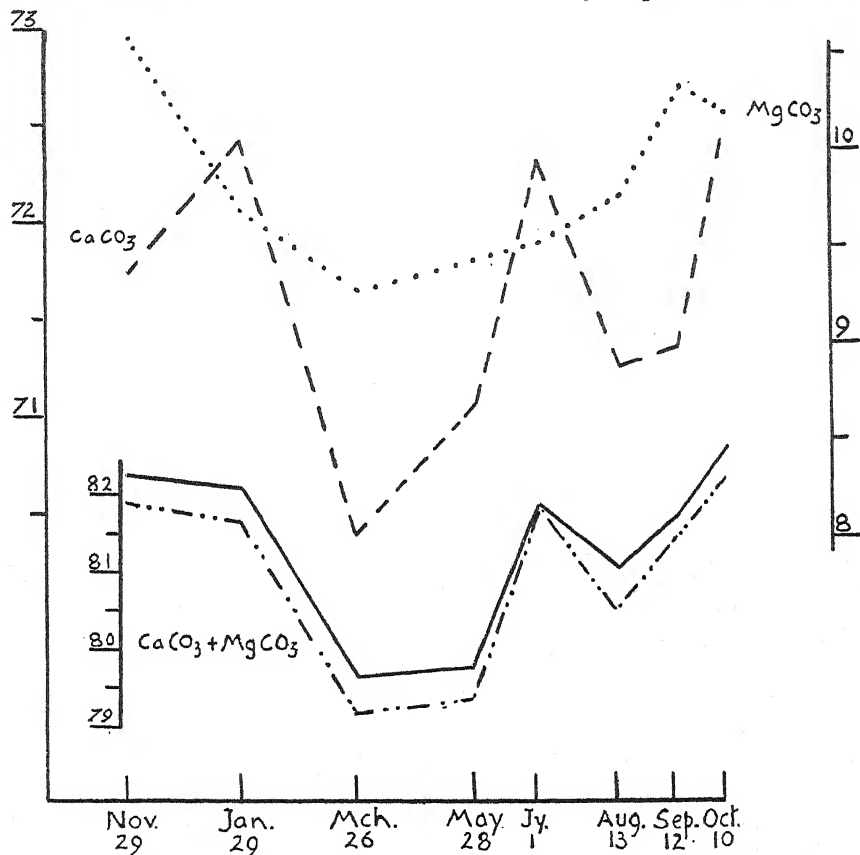


FIG. 5. Seasonal variation in calcium and magnesium carbonates: magnesium carbonate; calcium carbonate -----; summation of total carbonate ———; titration values of total carbonate -.-.-.-.

of time was effected. The contents of the titration flask were simply transferred to a large Kjeldahl flask, which was heated in a boiling-water bath until the solution had evaporated to a small volume, after which the estimation was proceeded with in the usual way.

To estimate the amino, amide, nitrate, and ammonia nitrogen, aqueous extracts of the plant were made by heating with water in a flask immersed in boiling water for a definite time. Five successive extracts of each sample were made, the extracts were combined, concentrated under reduced pressure, filtered, and made up to a known volume. The estimations were determined by micro-methods: ammonia by distillation with magnesium

oxide; amide by distillation with caustic soda, allowance being made for the ammonia nitrogen; amino nitrogen by van Slyke's method; and nitrate, after reduction, by Devarda alloy.

The estimations are mean percentages based on dry carbonate-free thallus; they are shown in Table II and are plotted in Fig. 3, where the values of amino and amide nitrogen have been multiplied by ten.

TABLE II.

Mean Percentage Values of the Total Nitrogen of the Thallus and of the Extract, the Distribution of Nitrogen, and the Amount of Floridoside.

	Total N. of thallus.	Total N. of extract.	Amino N.	Amide N.	NH ₃ .	NO ₃ .	Floridoside.
1933							
Sep. 27	4.613	1.116	0.2206	0.091	0.013	0.0046	3.50
Nov. 29	5.426	1.331	0.2922	0.113	0.016	0.0430	2.76
1934							
Jan. 29	5.406	1.300	0.2413	0.101	0.014	0.0665	2.27
Mar. 26	5.428	1.364	0.3093	0.143	0.014	0.0603	3.53
Apr. 22	5.397	1.715	0.3338	0.163	0.020	0.0179	3.79
May 28	4.678	0.962	0.2056	0.076	0.007	0	4.67
July 1	4.658	1.074	0.2610	0.074	0.006	0.0069	3.74
Aug. 13	4.078	0.838	0.1905	0.072	0.004	0.0035	4.26
Sep. 12	4.220	0.735	0.1519	0.074	0.014	0.0353	4.79
Oct. 10	5.110	1.010	0.2475	0.082	0	0.0469	3.61

FLORIDOSIDE.

For the estimation of the floridoside the samples were extracted with water five times on a boiling-water bath; the extracts were combined, evaporated to a small volume under reduced pressure, and cleared with colloidal iron, the precipitate being removed by centrifuging and filtration. The filtrate was hydrolysed by heating for three hours at 70° C. with 5 per cent. hydrochloric acid. The solution was then filtered and neutralized, and the reducing sugar estimated by Bertrand's method. The amount of reduction was calculated as galactose, from which the floridoside equivalent obtained by multiplying by the factor 1.41.

As before, the estimations are mean percentages based on the dry, carbonate-free weed; they are given in Table II and, plotted as galactose for convenience in making comparisons, in Fig. 6.

Although no definite conclusions can be made from these analyses, some comments are permissible.

The total nitrogen of the plant is highest and fairly constant during the winter; towards the end of April a gradual fall sets in, the lowest value being found in August, after which there is a rise to the winter. It will be observed from Fig. 6 that the total nitrogen of the extract is reasonably

constant during the winter, there is then a slight increase in April, a fall in May, a slight rise in June, followed by a gradual decline to September, after which there is a rise.

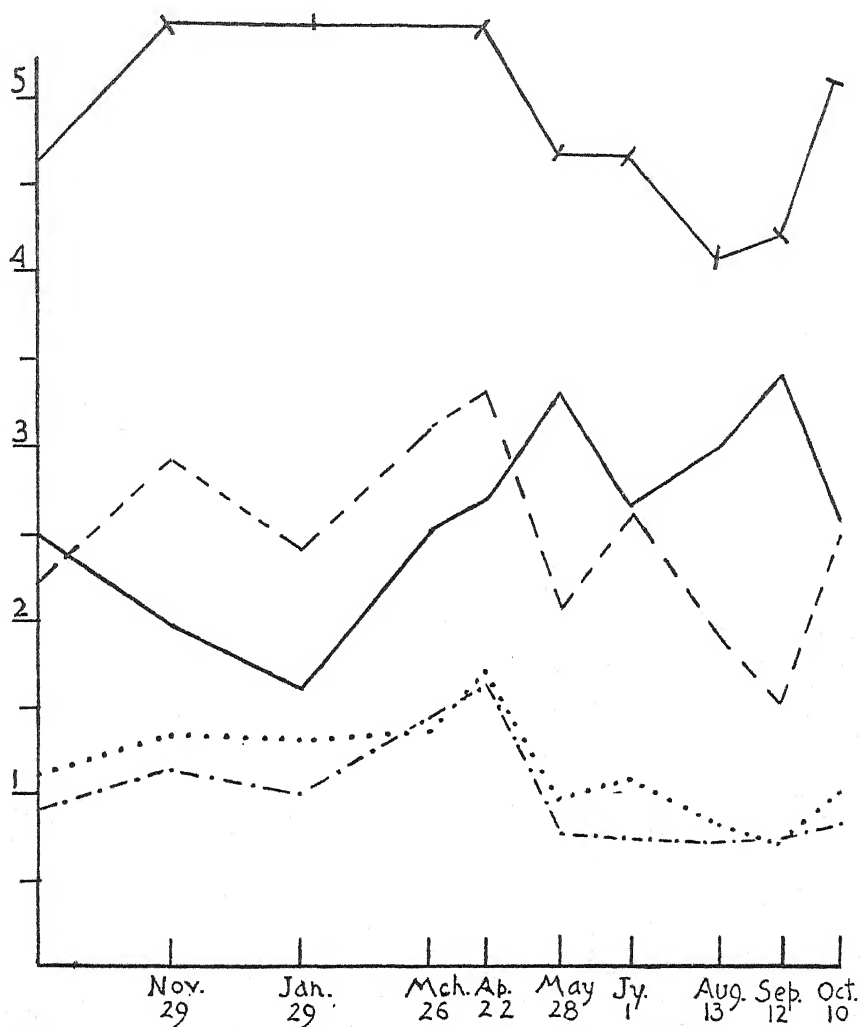


FIG. 6. Mean percentage values of total nitrogen of thallus +—+; total N of extract.....; amino N ($\times 10$)-----; amide N ($\times 10$).....; galactose —.

The amino nitrogen shows a wider variation; the curve shows an increase from September to November, followed by a decrease towards the end of January; there is then a gradual rise to the highest value towards the end of April. During May there is a fall, followed by a rise during June, when growth is most active; there is then a gradual fall to September, after which the amount increases.

It was originally hoped that the estimation of amino nitrogen would give the absolute value of the aspartic acid peptide, but it was found that the amount of amino nitrogen in the extracts was greater than could have been produced from the peptide alone. This indicates that the aqueous extract contains other amino compounds, the nature of which, although under investigation, has not yet been determined.

The amide nitrogen runs roughly parallel with the amino nitrogen to about the end of May, after which there is but little variation in its amount.

The nitrate content varies between wide limits, the highest values occurring in the winter, possibly associated with the seasonal increase of nitrate in the sea-water and the quiescence of the plant. In the growing season the amount of nitrate is low, and some correlation between it and the amino nitrogen can be traced: both are lowest in May, then a rise in July, followed by a fall in August, and finally a rise in the autumn. Whether this parallelism has any significance cannot yet be decided.

The ammonia nitrogen again is very small in amount and also shows a wide variation; no correlation between it and the other nitrogen fractions is obvious.

The amount of floridoside gradually falls from September to February, during which period light is limited and the plant is more or less dormant; it may be presumed that the loss of sugar is due to the respiratory needs of the plant. From February to the beginning of June, the month of most active growth, there is a rise; whilst during July there is a fall, due, no doubt, to the use of carbohydrate as a raw material in the making of new cell-walls. From July to September there is a gradual increase, followed by a fall which may be accounted for by the autumnal formation of new shoots. Compared with the amino nitrogen, there is a close parallel from the end of November to the end of April. After this, during the active period of the plant, there is a reciprocity; in May sugar rises and amino nitrogen falls, in June the reverse obtains, and a repetition of this occurs during the period July-October. So far, the conditions found during the period of activity support the working hypothesis that a low amino nitrogen would coincide with high sugar and vice versa, but, as it happens, it is at this period that the amounts of nitrate and ammonia are at their lowest, so that there is no increase in the total nitrogen. Clearly, the factors are too interwoven to admit of the elucidation of the problem by crop analyses; the evidence at least does not negative the hypothesis, but gives it some support.

One of us (W. K. H. K.) is indebted to the University of Leyden for a grant from the Vollenhoven foundation enabling him to take part in this investigation.

In conclusion, we wish to express our indebtedness to Mr. E. Rusbridge

of Worth Matravers for his help in collecting material, often in conditions of some risk.

SUMMARY.

1. The seasonal variation in the amount of incrustation, total nitrogen, the distribution of nitrogen, and the amount of floridoside in *Corallina squamata* is described.

2. The incrustation consists of calcium and magnesium carbonate and shows a seasonal variation, being highest in the winter and lowest in the spring. There is no constant ratio between the bases: the calcium shows two maxima, in January and July respectively; the magnesium is highest towards the end of November and lowest towards the end of March; its curve is smooth throughout the greater part of its course.

3. The nitrogen fractions show much seasonal variation; it is hardly possible to find a definite correlation between them and the total nitrogen.

4. Floridoside is at its highest amount towards the end of May and least at the end of January. During the active period of the life of the plant reciprocity between floridoside and amino nitrogen occurs; this gives some support to the working hypothesis that the constant presence of peptides in certain marine algae is due to a lack of balance between the carbon and nitrogen metabolism.

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NOTE.

ON THE GERMINATION OF *LEMANEA TORULOSA* IN CULTURE.—

There is still a considerable uncertainty about the complete life-history of this member of the freshwater *Rhodophyceae*. The observations made hitherto refer to

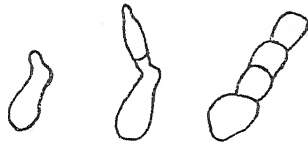


FIG. 1. Carpospores of *Lemanea torulosa*, early stages of germination. $\times 280$.

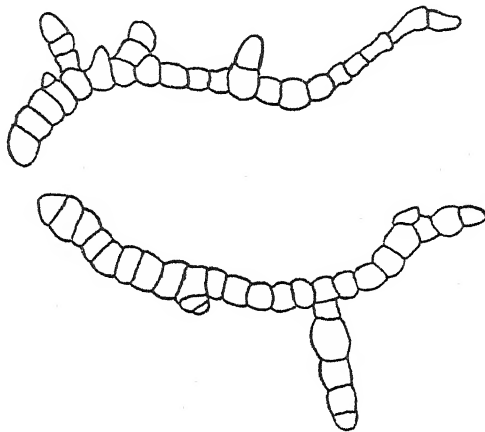


FIG. 2. Young sporplings of *Lemanea torulosa*. $\times 280$.

freshly collected material only, and so naturally leave a few gaps. Several examples in recent years show that in this field of study it is a great help, and sometimes a necessary condition for conclusive results, to grow the organism in question in culture in the laboratory. A special refined method has been worked out by various authors, and certain precautions must be taken to ensure successful growth. Purity of the medium both in the chemicals and distilled water used, the constitution of the glass-ware, and the effects of temperature and light are of chief importance.

A successful attempt has now been made to grow *Lemanea torulosa* in the laboratory. The material was collected from the rocky west shore of Lake Windermere. Loch Ness (according to Fritsch) is the only other place where this cold-stream alga has ever been found in still water. In both cases the definite wave action may

provide somewhat similar conditions to those found in rapidly flowing streams. It is an exception, however, and is not reported in the literature anywhere, for *Lemanea* to leave its original habitat of cold, rapidly flowing streams.

The alga was kept in Pyrex glass dishes in a special solution, containing the mineral salts MgSO_4 , K_2HPO_4 , NH_4NO_3 , CaCl_2 in a total concentration of 0.05 per cent. and 10 per cent. of a concentrated soil extract. Within a few days carpospores were formed and various stages in their formation could be observed. The spores were then isolated on agar-plates. This half-solid medium has been made up by adding 1.5 to 2 per cent. of fibre agar to the solution just mentioned. This solid medium has been found very useful for a number of algae of various groups. It has the advantage of facilitating the continued observation of the development of one particular spore and sporeling.

The spore is generally oblong or pear-shaped and new cells are formed at each end so that, usually, the original position of the spore is not recognizable in a later stage.

Fig. 1 shows the earliest stage of germination; Fig. 2 shows definite branched threads with typical 'chantransia cells'. The largest sporeling shows at present about 75 cells and is a branched thread, measuring $670\ \mu$ in length.

The method of germination of the carpospore suggests that meiosis does not occur at this stage. Further work is in progress on the possible occurrence of asexual spores on the chantransia stage, and on the cytology of the zygote, where meiosis is likely to occur. It is hoped that our knowledge of the life-history of *Lemanea* will thus be completed.

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Gametogenesis in *Allomyces arbuscula*.¹

BY

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With thirty-three Figures in the Text.

CONTENTS.

	PAGE
I. HISTORICAL INTRODUCTION	623
II. MATERIAL, CULTURAL METHODS AND CYTOLOGICAL TECHNIQUE	625
III. OBSERVATIONS ON LIVING MATERIAL	626
(a) Morphological considerations	626
(b) Cytological considerations	627
IV. OBSERVATIONS ON FIXED MATERIAL	637
V. DISCUSSION	643
VI. SUMMARY	647

I. HISTORICAL INTRODUCTION.

THE genus *Allomyces* was established in 1911 by Butler (3) to house a new member of the Blastocladiaceae which he collected in India. In this plant, which he named *A. arbuscula*, no evidence of sexuality was seen. The next year the same species was found in Ithaca, N.Y. by Barrett (2), who also described it as lacking in means of sexual reproduction. In 1922 Coker and Grant (5) isolated the same fungus at Chapel Hill, N.C., and since no sexual reproductive structures were discovered they were disposed to interpret certain resting sporangia as 'unfertilized eggs' that developed parthenogenetically. This fungus has been isolated from Mississippi soil by Harvey; and Lugg (12), 1929, studied material supplied by Harvey. A species of *Allomyces* has also been reported from the Philippines by Weston (18) and still another species, *A. moniliformis*, has been described from Smith Island, N.C. by Coker and Braxton (4). It was not until 1929, however, that sexuality was discovered in the genus. In that year Kniep (11) was able to distinguish gametangia, and to demonstrate the fusion of gametes in *A. javanicus*, a new species, which he discovered in Java.

¹ Botanical contribution from The Johns Hopkins University, No. 129.

Although the first published account of sexuality in *Allomyces*, that of Kniep, appeared in 1929, Weston (18) ten years before had observed in his Philippine species that among the 'zoosporangia' there was a regular alternation of large, ovoid to spherical 'sporangia', with small, cylindric to barrel-shaped 'sporangia', 'and that the larger sporangia were greyish-protoplasmic in colour, the small, barrel-shaped ones orange'. He made drawings of these 'sporangia' and 'zoospores', and at the top of one sheet of drawings he had written, 'Are these gametangia and gametes?' This question remained unanswered until Kniep published his article on the sexuality of *A. javanicus*.

Kniep's discovery of sexuality in *Allomyces* was epochal not only because it clearly demonstrated, for the first time, the occurrence of sexuality in the Blastocladiaceae, but because it was also the first case of heterogamous, planogametic conjugation known to exist among the Phycomycetes. In *A. javanicus* the male and female gametangia appear at the hyphal tips, the male commonly being terminal. The smaller, male gametangium is orange to brick-red; the female is grey or colourless. Both are of the same ellipsoidal shape. The male and female gametes are alike in that each is ellipsoidal and uniciliate. They are, however, different in size, the male gamete being about half the size of the female. These unciliate hetero-gametes conjugate to form biciliate plano-zygotes. The zygotes germinate in three to four hours, forming new plants. Kniep was unable, in living material, to follow the process of conjugation in its entirety, but he was able to demonstrate it cytologically. He found a complete series in which he could trace cytoplasmic and nuclear fusion.

The first published confirmation of Kniep's observations was made in 1933 when sexuality was described in *A. arbuscula* Hatch (10). In *A. arbuscula* the male and female gametangia are normally paired. They appear at the hyphal tips, the female terminal. The male and female gametangia differ in colour, shape, and size, the male gametangia being salmon-pink, barrel-shaped, and of smaller size, the female ashen grey, more nearly spherical, and notably larger. The gametes differ in colour, size, and motion. The male is of a brassy colour, the female grey. The male is one-half to one-third the size of the female, and shows a more active, darting motion than the female. Both are of the same spherical to subglobose shape. The conjugation of living gametes was described in detail.

Kniep considered *A. javanicus* of specific rank because it was the only species of *Allomyces* then known to be sexual. Although he used this distinction alone to separate it from *A. arbuscula*, *A. javanicus* still stands as a distinct species, even though sexuality has been discovered in *A. arbuscula*, because in *A. javanicus* the male gametangium is commonly terminal on the hypha, whereas in *A. arbuscula* it is the female that is regularly so. In this connexion it is interesting to note that Kniep isolated,

in addition to *A. javanicus*, another species of *Allomyces* that contrasted in its sexuality with *A. javanicus*, as does *A. arbuscula*. This 'Bali' species, when re-investigated, may very well prove to be *A. arbuscula*, or a variety of this species.

The curious and altogether remarkable type of sexuality described above deserves careful analysis. To this end a study of gametogenesis in *A. arbuscula* was inaugurated.

II. MATERIAL, CULTURAL METHODS, AND CYTOLOGICAL TECHNIQUE.

Material.

The material for this study was collected from a stream near Raleigh, N.C., July 7, 1931. Since that time it has been kept in culture in the Botanical Laboratory at the University of North Carolina, and it was from these cultures that mycelia were selected and brought to this laboratory by Prof. John N. Couch.

Cultural methods.

With the discovery of sexuality in *A. arbuscula* it became apparent that there are two types of mycelia, asexual mycelia bearing zoosporangia and resting sporangia, and sexual mycelia bearing gametangia and resting sporangia. The asexual mycelia are derived from zoospore or zygote isolations; the sexual mycelia from isolations of zoospores from resting sporangia formed on sexual or asexual mycelia. To obtain sexual mycelia the following method can be recommended:

1. Dry sterile agar cultures of either sexual or asexual mycelia at room temperature for a few weeks to several months, or dry at 40° to 45° C. for one to two hours.
2. To dried cultures add distilled water to soften agar.
3. Cut out 1 square centimetre of agar and inoculate hemp seed (4 halves of cotyledons) in aqueous culture.
4. Sexual mycelia will be ready to study or to fix in about three or four days.

This method, beside assuring the production of sexual mycelia, can be recommended because material can be stored in the dry condition indefinitely, cultures can be kept free of bacterial contamination, and the amount of inoculum can be controlled.

Cytological technique.

In the study of living material whole mycelia were used. After washing out the selected mycelia in fresh water they were either transferred to hanging drops or mounted under supported cover-slips. The change of conditions given by washing provides a strong impetus to gametogenesis, which can then be carefully followed under the high

powers of the microscope. The source of illumination, unless otherwise stated, was artificial light. Of the vital stains employed in this study Janus green and neutral red were most relied upon. Janus green was found specific for chondriosomes when used in the proper dilutions, and a technique employing neutral red was developed whereby differential staining of the vacuoles could be accomplished.

In the study of fixed material it was found both practicable and indeed advisable to use whole mycelia. A great many different fixatives and stains were tested for artifacts by constant comparison with the conditions obtaining in the living material. In the nuclear study the Feulgen reaction was found to be most adequate. In the investigation of the cytoplasmic inclusions several methods recommended themselves. For the lipoid granules an osmic acid technique proved most satisfactory. For the chondriosomes three methods were used: Regaud's, Meves', and a combination of Benda fixation and Champy-Kull staining. Of the three the Benda-Champy-Kull combination was probably the best; but all methods were used, the evidence from one supplementing that from the others. All the types of technique mentioned above are described in Lee's 'Microtometist's Vade-Mecum', ninth edition, with the exception of an adaptation of the Feulgen reaction for chromatin, which is herewith printed in full.¹

III. OBSERVATIONS ON LIVING MATERIAL.

(a) *Morphological considerations.*

In gametogenesis an important feature is the striking colour change that occurs in the male gametangium. Since the colours are more intense in daylight all observations on this particular phenomenon were made with this type of illumination. In the hyphal tips (Fig. 1) the concentrated cytoplasm appears grey-black in colour. When the gametangia are cut off from the hypha their contents are likewise grey-black. Later, both gametangia show a distinct bulging of their lateral walls, but there is no increase in diameter in the region of the cross walls, so that a definite constriction arises between the female and the male gametangium and a lesser one between the male gametangium and the hypha proper. At this stage the male gametangium lightens in colour, taking on a yellow tint. Still later, as the female gametangium continues to increase in diameter,

¹ The Feulgen reaction for chromatin is as follows: 1. Fix in Feulgen's fixative, 20 minutes. 2. Wash in distilled water through several changes. 3. Hydrolysis: Cold 0.5 N hydrochloric acid, 10 minutes. Hot 0.5 N hydrochloric acid, 58°C., 18 minutes. Cold 0.5 N hydrochloric acid, rinse. In the hot hydrochloric acid do not let the temperature rise above 60°C. 4. Stain in fuchsin-sulphurous acid, 1 hour. Glassware (vials) must be perfectly clean. Wash in cleaning solution and rinse well before using. 5. Wash in sulphurous acid, two changes. 6. Rinse in tap water. 7. Dehydrate in alcohol. 8. Clear in xylol. 9. In xylol, dissect mycelium from the hemp seed, wash out. 10. Mount in balsam. For the formulas of fixative, stain and other solutions, see Lee's 'Microtometist's Vade-Mecum', ninth edition, page 306.

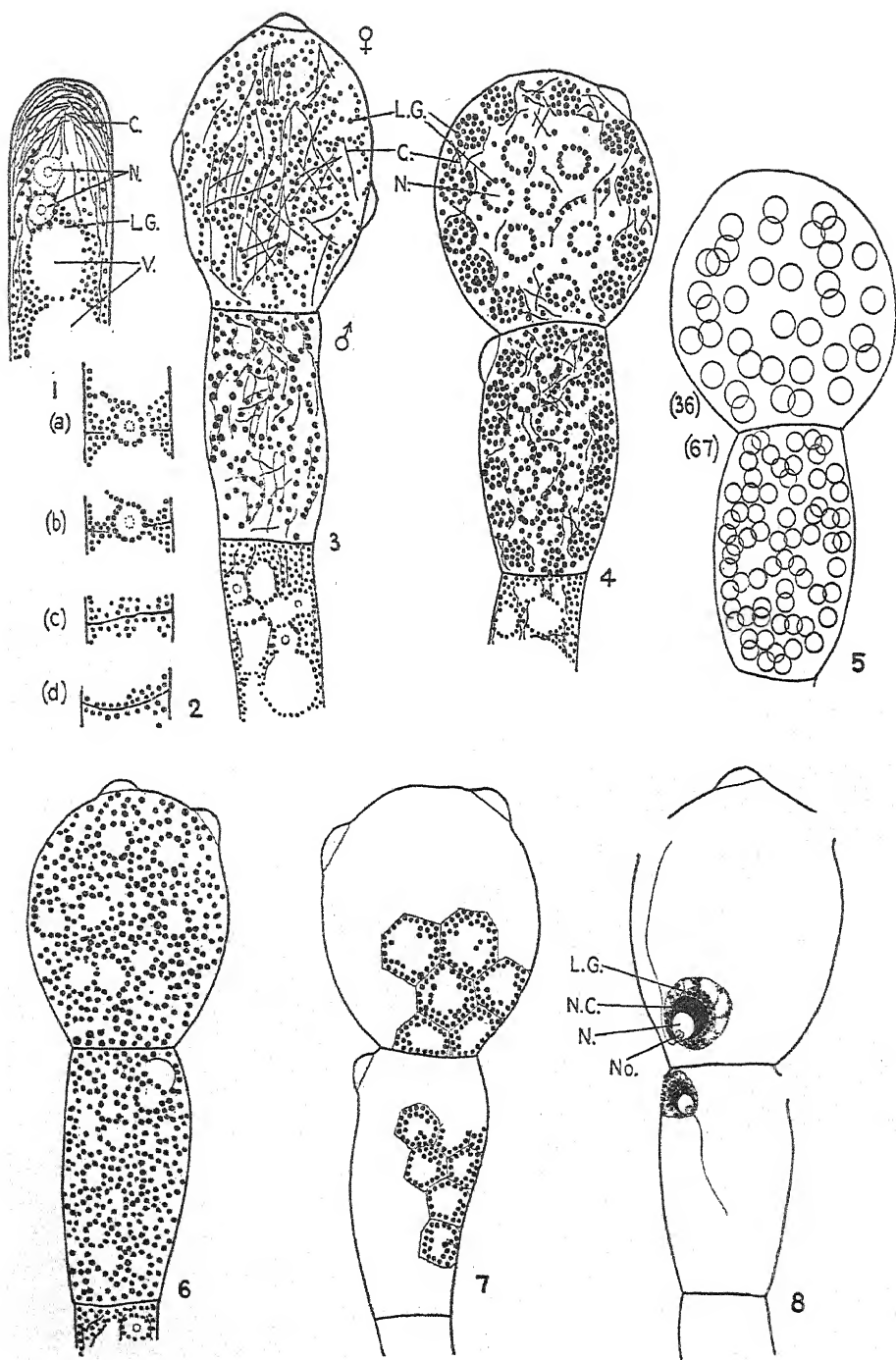
and to round off as it advances towards its mature, nearly spherical form, and as the male attains its mature barrel-shaped proportions, the male gametangium takes on a distinct rusty hue, while the female merely becomes lighter, assuming a dull grey colour. Papillae then appear on both gametangia (Fig. 3). With the appearance of these processes the male gametangium becomes light salmon-pink, the female dull grey. These colours, the pink for the male, and the grey for the female, are now maintained throughout the organization of the gametes (Figs. 4 and 6), and up until the time the gametes are delimited by cleavage furrows (Fig. 7), at which time the male gametangium loses its pink tint, and becomes brassy in colour. As the gametes emerge (Fig. 8) the intensity of colour in both male and female gametes is reduced through diffusion, but nevertheless a sharp distinction still exists in that the females are characteristically grey, the males typically of a pale brassy colour.

(b) *Cytological considerations.*

The Hyphal tips: Gametangia are formed at the ends of the hyphae by cross walls that come in just behind the hyphal tips. The protoplasm in such hyphal tips is very dense, contrasting sharply with the more vacuolate condition found elsewhere in the mycelium. In the proximal segments of a hypha a large, central vacuole runs the length of the segments. In segments nearer the tip the large central vacuole becomes smaller in diameter, the peripheral protoplasmic sheath thicker. In the most distal segment the central vacuole breaks up into several vacuoles, and these vacuoles become progressively smaller and more widely spaced toward the hyphal tip. The protoplasm consequently becomes more concentrated toward the tip.

The nuclei in the hyphal tip (Fig. 9) are very numerous, and follow, in their distribution, the concentration of the cytoplasm. They do not, however, move out into the tip itself. In living unstained material the nuclei appear as faint, grey, spherical bodies outlined by inclusions in the cytoplasm. A darker body in each nucleus, the nucleolus, helps greatly in the certain identification of the nuclei.

Of the inclusions in the cytoplasm the most obvious are the lipoid granules (Fig. 1). It is these structures that give the characteristic granular appearance to the cytoplasm. Massed though they may be in the hyphal tip, it is only a few that extend ahead of the last, most distal nucleus, and only a very few indeed that push into the tip itself. The lipoid granules are very small, but very distinct spheres, differing slightly in size. These granules show a restrained Brownian movement, oscillating persistently, but not moving through a large orbit. Either as a result of this movement, or from their inherent nature, or both, these lipoid granules aggregate about the larger, less mobile protoplasmic structures such as the nuclei,



FIGS. 1-8. Gametogenesis in living material. $\times 900$. 1. Hyphal tip vitally stained with Janus green showing distribution of chondriosomes and lipid granules. 2. *a, b, c, d*. Stages in gametangial cross-wall formation. 3. Male and female gametangia. The 'granular' stage of

and the plastids, and even line up along the chondriosomes. With artificial light these granules appear olive-grey in colour; with daylight, grey-black. They are, apparently, exactly the same type of granule as that which Guilliermond noted in *Saprolegnia* (7, 9) and *Leptomitus* (8), and which he described as 'lipoid granules'.

In among the lipid granules, and in turn reaching ahead of them, are the chondriosomes (Fig. 1). These structures become strikingly concentrated into a web of filaments that fits into the constricted hyphal tip. From this chondriosomal complex there depends peripherally a mantle of long chondriosomes. This mantle frays at its end, and merges gradually into the loose, peripheral arrangement of chondriosomes characteristic of the rest of the hypha. All the chondriosomes in this apical complex are filamentous, much attenuated, and very unstable in that they are capable of breaking up into short rods. The chondriosomes in all the more proximal segments of the hypha attain greater length and girth, and are so spaced that they can be readily distinguished the one from the other. Their long axes parallel the axis of the hypha. In the living, unstained condition, the chondriosomes appear light blue-grey. In a living hypha chondriosomes move sluggishly about, but whether this movement is due to cytoplasmic streaming, to the bombardment of lipid granules in Brownian movement, or in part to their own activity, cannot be said. Vital staining with Janus green reveals no new structures, but stains chondriosomes specifically, and so helps to identify chondriosomes and chondriosomal derivatives more definitely. The chondriosomes of *A. arbuscula* have much the same appearance as those described by Guilliermond for *Leptomitus* (8) and *Saprolegnia* (7, 9).

Cytoplasmic streaming is very important in bringing about this concentration of the nuclei, the lipid granules, and the chondriosomes at the hyphal tip. These three constituents can be seen moving up the hypha, and can even be seen threading the pseudosepta. This movement is most apparent in the lipid granules, which are to be observed constantly moving up along the inner face of the peripheral sheath of cytoplasm.

When gametangia are formed, two cross walls come in across just such a hyphal tip as that described above. These walls, unlike the perforate pseudosepta, are complete. They are formed by the ingrowth of two ridges that appear on the inner wall of the hypha. These ridges develop into thin diaphragms which finally close across the lumen of the hypha to

gametogenesis. Chondriosomes and lipid granules widely scattered. 4. Male and female gametangia. The 'gamete origin' stage of gametogenesis. Nuclei displaced peripherally, outlined by lipid granules. Chondriosomes in the interstitial cytoplasm. 5. 'Gamete origin' stage showing relative number and size of nuclei in male and female gametangia. 6. 'Disappearance' stage. Lipid granules scattered. Outline of nuclei lost. Chondriosomes no longer apparent. 7. Cleavage. Lipid granules alone apparent and these displaced peripherally in the individual gametes. 8. Male and female gametes each with a nucleus, a nuclear cap, and lipid granules. C. = chondriosomes; L.G. = lipid granules; N. = nucleus; NO. = nucleolus; N.C. = nuclear cap; V. = vacuole.

form the two cross walls. When a nucleus lies directly in the path of one of these walls the wall apparently makes a detour around the nucleus, with the result that it is concave toward the nucleus. For stages in the formation of one of these cross walls, see Fig. 2 *a, b, c, d*. These two cross walls are formed at about the same time, but they are not initiated simultaneously. In a couplet of very young gametangia the wall cutting off the terminal cell or female gametangium is thicker than that which cuts off the subterminal cell or male gametangium. Furthermore, instances may be found in still younger couplets where the wall cutting off the male is not complete, when that of the female is quite complete.

Gametogenesis, 'granular' stage (Fig. 3).

The diffuse, granular appearance characteristic of the gametangia when they are cut off from the hypha is, of course, to be attributed to a dispersed distribution of lipoid granules. Here and there, but particularly toward the surface, the granules may clump together, but for the most part they are strung out through the cytoplasm in an irregular network. In this initial stage of gametogenesis the lipoid granules are of the same size, and show the same Brownian movement as the lipoid granules in the hyphal tip; and irrespective of whether they have been segregated in a male or a female gametangium they are of the same grey-black colour.

The chondriosomes, like the lipoid granules, are widely scattered. They, too, are more numerous peripherally where they lie scattered among the lipoid granules.

The vacuolate condition of the cytoplasm noted in the hypha persists in the gametangia; vacuoles are numerous and well scattered through the gametangia.

The nuclei throughout the 'granular' stage of gametogenesis are found rather deep in the gametangia, where the lipoid granules are so dispersed that they cannot effectively outline them. The greater concentration of the lipoid granules peripheral to the nuclei tends only to screen the nuclei. The nuclei in the granular stage are, consequently, far from apparent and can only be identified after careful study. Despite these difficulties, however, enough can be seen to show that when the two cross walls close across the hypha the nuclei of the hypha are segregated to the male and the female gametangium in about equal numbers. The number of nuclei in each gametangium is increased almost immediately by nuclear division. Prior to these nuclear divisions the nuclei are irregularly distributed throughout the gametangia. After these divisions the nuclei become very evenly spaced.

The 'granular' stage is a stage in which the full nuclear complement of the gametangium is achieved. Furthermore, it is a stage in which the nuclei become so distributed that when called upon to act as gamete

organization centres, each has an equal and equivalent sphere of influence in a cytoplasm which is as yet quite unorganized. Finally, it is the stage in which the mature shape of the gametangium is attained, and the first colour change is initiated in the male.

Gametogenesis, 'gamete origin' stage (Fig. 4).

Soon after the mature form of the gametangium has been attained emergence papillae put in their appearance. With this new morphological development, striking cytological changes are initiated within the gametangium. The nuclei, heretofore well removed from the gametangial walls, now all become displaced peripherally. The lipoid granules once widely dispersed follow the nuclei to the walls of the gametangium and line up about them. These nuclei with their sheaths of lipoid granules constitute the 'gamete origins'. At first the lipoid granules at the surface of the gametangium are too few to ensheath the nuclei completely, but their number is gradually augmented as more and more lipoid granules move out of the central cytoplasm. As soon as the lipoid granules approach the surface they scatter among the nuclei. Here their erratic movements carry them about among the nuclei and ultimately bring them in contact with a nucleus where they take up their position on the nuclear membrane. In addition to this new distribution of the lipoid granules this stage of gametogenesis has brought significant changes to the lipoid granules themselves. They are larger now, larger than they were in the 'granular' stage. They have also changed colour. This change has been most spectacular in the male gametangium where the lipoid granules have acquired a colour which is yellow in artificial light, and salmon-pink in daylight. In the female gametangium there has been no such marked colour change in the lipoid granules; they may have lightened in colour, but they are still grey (in either light).

The 'gamete origin' stage is the most favourable stage of gametogenesis in which to study the chondriosomes. At this time the chondriosomes are concentrated peripherally in the interstitial cytoplasm between the nuclei. With the concentration of the lipoid granules about the nuclei a clear view of this interstitial cytoplasm is afforded. The fine, blue-grey chondriosomes can be seen most advantageously in the male gametangium where the yellow lipoid granules present a good contrast.

Vacuolar changes apparently account for the striking peripheral displacement of nuclei and cytoplasm that distinguishes this stage. The vacuoles, scattered in the 'granular' stage, crowd toward the centre of the gametangium where they fuse into several larger vacuoles or a single, large, central vacuole. In the female gametangium this vacuolar system may occasionally be long and tortuous, but more commonly it is contracted toward the centre of the gametangium, and only pushes out a few blunt lobes into the peripheral cytoplasm. In the male the vacuoles fuse into

a more ramifying vacuolar system. This system is gathered toward the centre of the gametangium and extends from one end to the other.

Comparing the male and female gametangia at this stage of their development, it is apparent that there is just as striking a contrast in their respective nuclei as in the colour of their lipid granules. It will be recalled that during the 'granular' stage there were numerous nuclear divisions in the young gametangia. It now appears that there must have been approximately twice as many mitoses in the male as in the female gametangium, for there are roughly twice as many nuclei in the male as in the female (Fig. 5). Furthermore, the nuclei of the male gametangium are but one-half the size of the nuclei of the female. No difficulty is experienced in studying the nuclei at this stage. Their extreme peripheral position and their definite outline makes their study easy. Even the nucleoli can be readily seen. A word ought to be said here also about the mobility of the nuclei. In this stage the nuclei are constantly changing position as a result of the jostling of the lipid granules. This drifting of the nuclei is, however, to be noted at all stages of gametogenesis, so it is apparently of no particular significance. There are, however, other major nuclear displacements that are significant, such as that involved in their recent peripheral migration.

In the first or 'granular' stage of gametogenesis the nuclei become evenly dispersed throughout the cytoplasm of the gametangia. In this second or 'gamete origin' stage these nuclei serve as organization centres for the cytoplasmic constituents, drawing about themselves, first, a sheath of lipid granules, then a tangle of chondriosomes.

Gametogenesis, 'disappearance' stage (Fig. 6).

Gametogenesis is often temporarily arrested in the 'gamete origin' stage. In old cultures almost all gametangia will be found to have reached this stage of development, there to await a change of conditions before completing the organization of the gametes. In practice, the only change of conditions found necessary was a change of water. This brought about a completion of gametogenesis within the hour. The first sign of renewed activity in such arrested gametangia is the crowding of the lipid granules into the cytoplasm between the nuclei. Ultimately a heavy screen of lipid granules is drawn over the whole surface of the gametangium, and the lipid granules become noticeably motionless. The gametangia do not remain in this state of organization for any length of time, however, because very shortly the 'gamete origins' sink toward the centre of the gametangium. In this crowding of the 'origins' towards the centre of the gametangium the lipid granules gradually become widely dispersed and the nuclei, once so distinct, become less and less easy to identify until ultimately all that can be identified as nuclei are the numerous clear areas appearing among

the lipid granules. Thus the gametangia, for all their erstwhile organization, appear again superficially quite unorganized. The 'gamete origins' pass into a 'disappearance' stage.

Changes in the central vacuole apparently account for the central displacement of the 'gamete origins'. Back in the 'gamete origin' stage the central vacuole or vacuoles begin to send out peripheral processes. In the 'disappearance' stage the further conversion of the large central vacuole or vacuoles into a system of vacuolar wedges or cleavage furrows results in the displacement of the cytoplasm toward the centre of the gametangium. At the very outset of this cytoplasmic migration the nuclei abandoned their peripheral location for a more central position on or near the central vacuole. With the centrifugal progress of the vacuolar wedges the nuclei are left behind, each in a sort of cradle between the advancing wedges. The chondriosomes cannot be identified at this stage of gametogenesis.

Gametogenesis, cleavage (Fig. 7).

In living unstained material it is very difficult to follow the progress of the vacuolar wedges or cleavage furrows. In mycelia vitally stained with neutral red, however, the peripheral advance of the vacuolar cleavage furrows can be demonstrated. All through the 'disappearance' stage these cleavage furrows can be seen slowly pushing out through the peripheral cytoplasm. At their advancing tips the cleavage furrows are drawn out into thin, tortuous channels. Before the vacuolar wedges have had time to push to the wall of the gametangia the first gametes, those that occupy the centre of the gametangia, have already been cut out. A sort of shudder goes through the gametangium as these furrows come in. The nuclei during cleavage maintain a position deep in the gametangium where they abut on the central vacuolar system. As cleavage proceeds the lipid granules line up along the cleavage furrow. Although there is considerable movement among the lipid granules at this time it is, of course, restricted in that the granules can only move within the limits set by the cleavage furrows.

After cleavage has been accomplished the fate of the chondriosomes can at length be determined. It is apparent that they have undergone a striking metamorphosis, for they now appear as one, two, or three large blobs which closely invest the nucleus. As the cleaved origins, now gametes, further individualize themselves, these several chondriosomal blobs fuse until but one large one remains; this mass subsequently becomes applied to the nuclear membrane forming a sort of nuclear cap (Fig. 8).

The Gametes (Fig. 8).

Normally, the discharge of the gametes begins soon after cleavage has been completed, but in the interval before discharge there are incipient movements among the gametes within the gametangia. These movements are most apparent among the gametes that abut on the emergence

papillae, for among them there is a very definite crowding and pushing as they force their way into the papillae. After the bursting of the papillae of emergence by the first gametes there is a rather steady egress of gametes, but as the gametangia are gradually emptied the interval between the escape of the gametes at any one pore becomes longer. Thus, while the majority may have escaped in the first 10 minutes, a few (1 to 3 or more) will still be found in the gametangium 20 to 30 minutes later. Sometimes the two gametangia of a couplet may discharge almost simultaneously, but more often the male precedes the female in dehiscence, and this despite the fact that the male was cut off from the hypha somewhat later than the female.

The male gametes appear one at a time sometimes close behind each other but nevertheless always distinct. In making their escape they have first to push through a small pore the narrowness of which constricts them in the process. But even when its body is outside the gamete is not always free, since frequently it has trouble in disengaging its long cilium which it drags behind while pushing out of the gametangium. Indeed, four hapless gametes have been seen to tug for 14 minutes before they could free their cilia.

The female gametes are slow to escape. In some instances they are completely individualized when they push through the pore of the papilla. Once free of the gametangium, however, they are at first perfectly quiet. Then they begin to swing slowly on their axes through an arc of not more than 45° . This movement ultimately gives way to a rapid vibratory motion, and finally the gametes move off, each propelled by its single, posteriorly attached cilium. At other times the female gametes escape in blobs that are apparently undifferentiated and certainly remain quite motionless for a time. As the separate gametes gradually attain their individuality, rounding up and separating from the mass, it appears that as many as ten to twelve gametes may be contained in such blobs.

In the gametes all essential structures can be seen clearly (Fig. 8). Both male and female gametes are spherical to subglobose in shape, and although instances of biciliated gametes may be noted in some cultures, the gametes, male or female, have characteristically but a single, long, posteriorly-attached cilium. At the base of this cilium and at the point where the cilium touches the membrane there is a very small granule. Supplementing these observations on living gametes (Fig. 8) by a study of fixed gametes (Fig. 20) it appears that this granule, which marks the base of the cilium, is connected with the nucleus by two fine filaments. The nucleus in the gametes, as has been stated, is closely invested by a bulky nuclear cap; together these bodies form a conspicuous pyramidal structure the apex of which is directed toward the posterior end of the gamete. Since the nucleus is situated at the apex of this pyramid and since the compound

structure has been displaced posteriorly the nucleus is brought close to the gamete membrane just opposite the point of attachment of the cilium. A nucleolus is always to be found lodged against the nuclear membrane at the point at which the nucleus lies in closest proximity to the basal granule of the cilium. The two filaments which extend from the base of the cilium to the nucleus contact the nuclear membrane at two points, to the right and left of the nucleolus. These points mark the farthest extension of the chromatic reticulum about the nucleolus. In a motile gamete the two arms of the chromatic reticulum are being constantly stretched and relaxed by the movements of the nucleolus. At one time the nucleolus strikingly extends the nuclear membrane, then it momentarily sinks back into the nucleus before it again distends the membrane and so repeats the process. The nucleolus also experiences lateral displacements. Where this movement is initiated is not known, but certainly this part of the nucleus is always showing distortion.

When the gamete is in motion the nuclear cap and the imbedded nucleus act as a unit; any displacements of the one affect the other. About the nucleus and the nuclear cap the cytoplasm is dense, whereas in the peripheral regions of the gamete the cytoplasm is strongly vacuolate. The lipid granules are generally to be found in the denser cytoplasmic region, and particularly in the cytoplasm that immediately invests the nucleus and nuclear cap. As the gamete changes the character of its movement, the lipid granules slide around on the nuclear cap to new positions. When the gamete is swimming they usually become concentrated toward the anterior end of the cell. With the cessation of active movement, at least some of the lipid granules glide back down and around the nuclear cap and even gather behind the nucleus.

When the gametes cease swarming, amoeboid movements begin, and a hyaline pseudopodium is extended, the lipid granules quickly crowd in behind the hyaline cap. The nuclear cap then moves over and becomes wedged into the base of the pseudopodium, and as soon as the major portion of the cap has swung into the pseudopodium the nucleus and its attached cilium is pulled after it. In making this passage the nuclear cap is not greatly altered in form. After the passage of the nuclear cap and nucleus into the pseudopodium the body of the gamete quickly follows.

Both the male and the female gametes, as has already been noted, are spherical or subglobose in the free swimming condition; both are uniciliate. They are also alike in internal organization so that the above detailed description applies to both male and female gametes. The gametes differ, however, in many salient respects (Fig. 8). First, the female is two to three times as large as the male. Second, the female is grey in colour, the male is of the colour of brass, these being the colours of their lipid granules. Third, the nuclei in the female are twice as large as those in the

male. Fourth, the volume of the nuclear cap in the female is three to four times as great as in the male. Finally, the male gametes, on the whole, are more active than the female; they become active in the gametangium itself and can be seen moving around jerkily as soon as partial discharge permits.

As might be expected, the general vigour of the gametes differs in different gametangia, and particularly in different cultures. Hence there is no agreement in the duration of their swarming period or in their activity during that period. The duration of the swarming period for females that fail to mate was found to be short, however. In one extreme case the female gametes issued from the gametangium very slowly and they were more than ordinarily delayed in individualizing themselves from the mass in which they escaped. Once free, their cilia moved but weakly and they came to rest almost immediately without conjugating and without leaving the field of a 4 mm. objective. In most cultures the females show vigorous ciliary motion and remain active for a longer period. The male gametes, in the best example that could be held under observation, came to rest in twenty-two minutes, without going out of the field of the 16 mm. objective. Observations on the motion of the gametes are not in complete agreement. When in close confinement, the motion is different from that when free-swimming. When gametangia are hemmed in by hyphae and detritus the male gametes exhibit a darting movement. The gametes dart forward for a short distance and then vibrate, holding approximately the same position; this procedure is then repeated. For the sharp darting movement the cilium, which has swung forward in a loose loop, is snapped back; then, held hard back, it is whipped around in small circles, thus imparting a vibratory or 'wriggling' motion to the gamete itself. The movement of the female gamete when similarly restricted is like that of the male but less rapid.

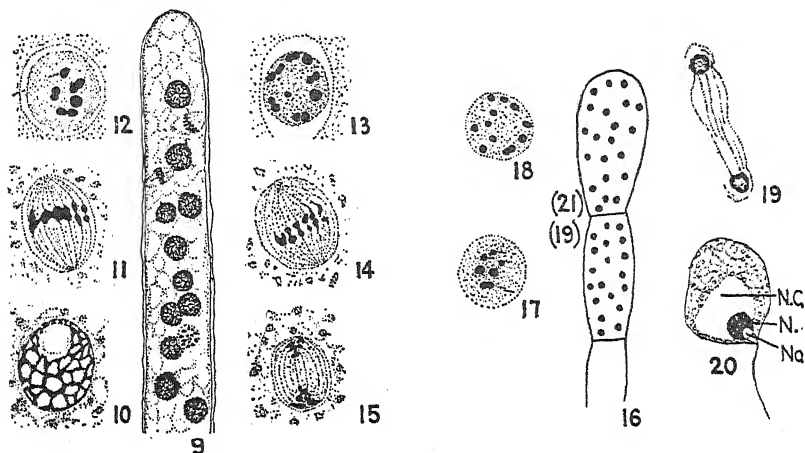
When a gamete is in the open and its movement is unrestricted it glides steadily along; it also apparently turns on its axis as it moves. This is especially evident in a free-swimming zygote, on which a slight bulge marks the position of the male. This irregularity of contour helps to establish the rotary movement of the zygote on its own axis.

The gametes also have the power of amoeboid movement (p. 635); this is particularly marked in the female and is not dependent upon fusion. In certain unfused female gametes which abandoned active ciliary movement and came to rest soon after their discharge, striking amoeboid movement was initiated in about twenty minutes.

IV. OBSERVATIONS ON FIXED MATERIAL.

The hyphal tips.

Observations on the nature and distribution of the cytoplasm and the cytoplasmic inclusions made with fixed material serve only to confirm those made on living material (see Figs. 21 and 22 for chondriosomes and lipoid granules). They do, however, provide additional information



FIGS. 9-15. Nuclear phenomena in the hyphal tip as demonstrated with the Feulgen reaction for chromatin. 9. Hyphal tip showing distribution of nuclei. Three nuclei in mitosis. $\times 1,200$. 10. A resting nucleus. $\times 3,200$. 11. Early anaphase, side view. Chromosomes beginning to split. $\times 3,200$. 12. Early anaphase, polar view. Chromosomes beginning to pull apart $\times 3,200$. 13. Anaphase, polar view. Chromosomes already separated. $\times 3,200$. 14. Anaphase side view. $\times 3,200$. 15. Telophase. $\times 3,200$. Chromosome count six. Compare Figs. 11-15.

FIGS. 16-20. Nuclear phenomena in the gametangia as demonstrated with the Feulgen reaction for chromatin. 16. Young gametangia but recently cut off from the hypha showing that the nuclear complement of the female gametangium at this time may even be greater than that of the male. $\times 800$. 17. Anaphase, polar view. Chromosomes beginning to pull apart. $\times 3,200$. 18. Anaphase, polar view. Chromosomes separated. $\times 3,200$. 19. Telophase. Spindle stretched and collapsed. Chromosome groups pulling apart. About $\times 2,000$. Chromosome number six, same as in hyphal tip. For counts in gametangia compare Figs. 17, 18, and 19. 20. A female gamete. Chromatic reticulum of the nucleus staining. Nucleolus and nuclear cap unstained. $\times 1,200$. N. = nucleus; NO. = nucleolus; N.C. = nuclear cap.

concerning the nuclei. These, in the terminal segments of the hyphae, are very numerous and are scattered at random in the cytoplasm (Fig. 9). At least one or two of these nuclei are likely to be in mitosis. Only in young reproductive structures is the proportion of the mitosis to the total number of nuclei greater. The chromosome number is six. For mitotic figures and chromosome counts see Figs. 10-15.

Gametogenesis, the 'granular' stage.

When cross walls come in across the hyphal tip the nuclei, being irregularly distributed are segregated to the male and female gametangia in about equal numbers (Fig. 16). The gametangia are thus multi-nucleate from the very beginning, cross-walls in heavily nucleated hyphae necessarily cut out cells with a fairly full complement of nuclei.

Furthermore, these nuclei when they are first separated from the rest of the nuclei in the hypha are of the same size as the nuclei below them in the hypha. As soon, however, as these two gametangia, so alike in their nuclear constitution, are separated by a cross wall they show a difference in their mitotic behaviour. Although the male gametangium is a smaller structure, and although it is at first fully as well supplied with nuclei as the female, the nuclei of the male undergo approximately twice as many mitoses as those of the female. Nuclear counts were made in male and female gametangia when all mitotic activity had ceased. In one of the couplets selected for this study the male and female gametangia were of the same length. Since, however, the female was ovoid while the male was roughly cylindrical in shape, the volume of the female was somewhat greater. There were nevertheless 23 nuclei in the female, 40 in the male. In other couplets the counts were 12 female to 24 male, 36 female to 67 male (Fig. 5). The number of nuclei differs with the gametangia, but always there is this same approximate two to one ratio. In addition to this numerical difference, and probably in consequence of it, the male nuclei are one-half the size of the female nuclei (Fig. 5). The nuclear-plasmic ratio in the young gametangium is, of course, like that in the hypha, and in spite of the nuclear divisions subsequently occurring in the gametangium it cannot be demonstrated that this ratio itself undergoes any change. The change is in the volume of the individual nuclei. Those in the male lose as much as half their volume, those in the female only a fraction of that. There is, of course, some slight growth in the gametangia as is indicated by the bulging of the lateral walls, and there is a concentration of the cytoplasm. This of itself would allow of some multiplication of nuclei within the ratio.

While these nuclear divisions are in progress the nuclei are scattered at random throughout the gametangia (Fig. 16). These divisions are never simultaneous; only three to four at most will be found in mitosis in the same gametangium at any one time. The precise spacing of the nuclei throughout the gametangia achieved toward the end of the 'granular' stage is in part accomplished during the telophase of these mitoses. It is not at all uncommon to see the chromosome groups at telophase pulling apart for as much as half the diameter of the gametangium (Fig. 19). In this process the stretched spindle can be clearly seen. Chromosome counts made in both male and female gametangia show that the number remains the same as that in the hypha, namely, six (Figs. 17, 18). Thus, the possibility that a reduction division occurs at gametogenesis is eliminated.

The vacuolization of the cytoplasm and the distribution of the lipoid granules and chondriosomes in the 'granular' stage is found in fixed material to be the same as that in living material. Vacuoles are numerous and are widely scattered. Lipoid granules are irregularly and widely

scattered, some few lining up on the nuclear membrane. The chondriosomes consist of longer or shorter filaments, but even the longer filaments are only a quarter of the length they attain in the hypha (cf. Fig. 23 and Figs. 21 and 22). These filaments are scattered through the gametangia and lie in between and around and about the nuclei (Fig. 23).

Gametogenesis, 'gamete origin' stage.

In the 'gamete origin' stage the nuclei, as has been described, migrate peripherally to line up against the gametangial wall. The lipid granules follow the nuclei to the surface and line up about them, forming a close investment or sheath. In a Champy-Kull preparation (Fig. 24), however, only the skeleton of these lipid granules stains. Now the chondriosomes also follow the nuclei to the surface and become scattered between them (Fig. 24). Throughout the 'granular' stage the chondriosomes have been fragmenting, so that in the 'gamete origin' stage they take the form of very fine and relatively short filaments.

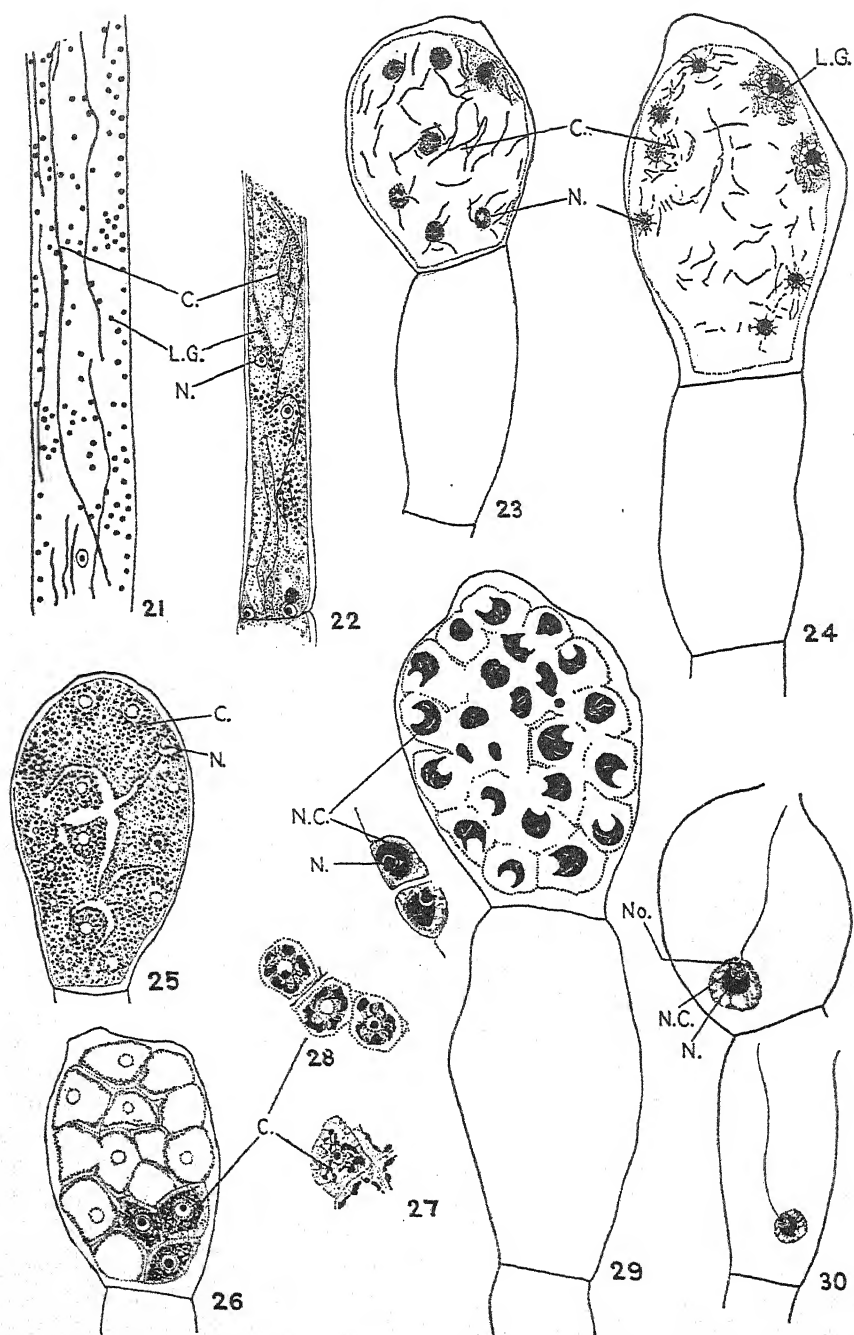
In the further progress of gametogenesis the important feature is the organization of the nuclear cap. This process could not be followed at all adequately in the living condition, so it became necessary to use special chondriosomal technique to follow the process through the critical 'disappearance' stage.

Gametogenesis 'disappearance' stage.

When the nuclei with their sheaths of lipid granules sink toward the centre of the gametangium the chondriosomes have fragmented and exist only as very short rods or granules; they move with the nuclei but remain peripheral to the sheaths of lipid granules that still invest the nuclei.

Gametogenesis, cleavage.

As the cleavage furrows push through to the gametangial wall a cloud of chondriosomes is cut off about each nucleus (Fig. 25). With cleavage completed, these minute chondriosomes begin to enlarge (i.e. become vesiculated) and so gain in distinctness (Figs. 26 and 31). Thus each nucleus, probably with most of its lipid sheath still intact, becomes buried in a mass of chondriosomal granules that must be apparent to even the casual observer. With the further vesiculation of these chondriosomal granules, contiguous chondriosomes coalesce (Fig. 27) to form a reticulum. This chondriosomal reticulum is thrown up about the nucleus in the cytoplasm toward the peripheral limits of the gamete. With further vesiculation the nucleus becomes enveloped in a relatively heavy but ragged mantle (Fig. 28). This mantle now begins to close in on the nucleus. After this manner, then, there is formed one rather large homogeneous mass of indefinite shape and dimensions which, as it invests the nucleus more closely tends to displace the lipid granules from the nucleus. The final disposition of this



FIGS. 21-30. Cytoplasmic phenomena of gametogenesis as demonstrated with Champy-Kull's chondriosomal technique. 21 and 22. Segments of the hypha. $\times 800$. 21. A segment toward

chondriosomal structure cannot be described satisfactorily until the developments in the rest of the gametangium are understood.

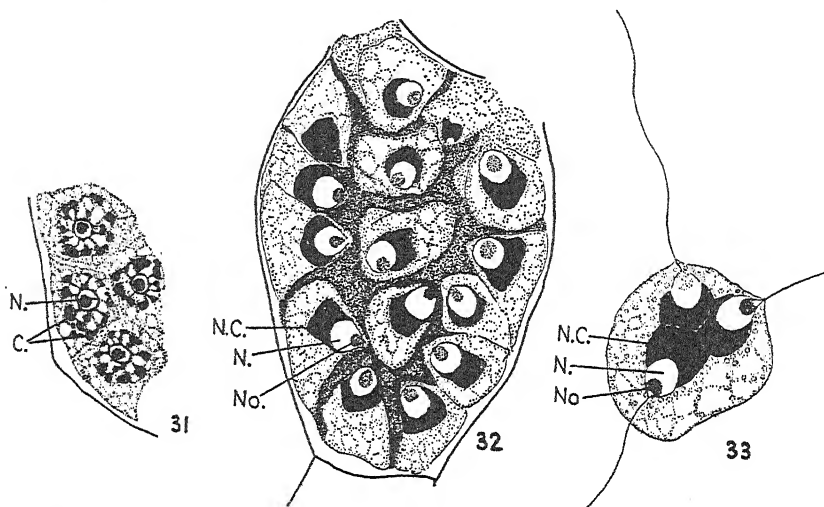
The cleavage furrows, as noted above, first appear in the centre of the gametangium, and gradually work out towards the periphery, cutting out the gametes as they progress (Fig. 25). The gametes in the centre, always relatively few in number, often lie completely separated from their fellows before the peripherally moving furrows have delimited the greater mass of gametes that line the wall. A cross section of a gametangium at this stage would show a single gamete completely delimited in the centre. Cleavage furrows would appear as narrow radiating wedges pushing out to the gametangial wall. Each of the peripheral gametes would appear triangular in a cross section of the gametangium. In three dimensions the shape of one of these peripheral gametes is that of a truncated pyramid with a hexagonal base.

In this process of cleavage the essential thing is apparently that each chondriosomal complex (nuclear cap) should be completely delimited. When the gametes are cut out in a normal gametangium each gamete has but a single cilium, a single nucleus and a single nuclear cap. Under abnormal conditions, however, the cleavage of the cytoplasm may be imperfect. Thus, in the case of one female gametangium three monstrous gametes were discharged along with normal ones. One (Fig. 33) possessed three cilia, three nuclei, and three nuclear caps; the second was roughly of the same size as the first, but it had neither cilia, nuclei, nor nuclear caps; the third was biciliate with two nuclei and two nuclear caps. In a gametangium that produces such monstrosities it is apparent that the process of gamete cleavage has been seriously disturbed. In the three-ciliated gamete the three nuclear caps were joined by their bases; the major portion of each of them, however, stood free of its neighbour. The nuclei stood free as at the apices of three triangles. In the biciliated gamete the same condition obtained: the nuclei were separated, but the nuclear caps joined. Thus it would seem that in the delimitation of gametes the essential thing is that the cleavage furrows should push through and separate contiguous chondriosomal complexes or nuclear caps.

A careful study of any one of the peripheral gametes (Fig. 32) shows

the base. 22. A segment toward the apex. Both show the great length attained by chondriosomes in the hypha. 23. 'Granular' stage of gametogenesis. Nuclei distributed throughout gametangium. Chondriosomes scattered and shorter than those in hyphae. $\times 1,200$. 24. 'Gamete origin' stage. Nuclei displaced peripherally and outlined by the skeletons of lipid granules. Chondriosomes, short filaments, concentrated peripherally. $\times 1,200$. 25. Female gametangium, 'disappearance' stage. Cleavage furrows formed. Chondriosomes granular. $\times 1,200$. 26. Female gametangium, cleavage completed. Chondriosomes drawn more closely about the nuclei. $\times 1,200$. 27. A gamete from a female gametangium. Chondriosomes enlarging and fusing into a ragged reticulum. $\times 1,200$. 28. Gametes from a female gametangium. Chondriosomal complex organized into a heavy reticulum. $\times 1,200$. 29. Gametangia and insert from female gametangium. Chondriosomal reticulum drawn together into a nuclear cap. $\times 1,200$. 30. Male and female gametes. Nuclear caps staining distinctly. C. = chondriosomes; L.G. = lipid granule; N. = nucleus; N.C. = nuclear cap.

that the nucleus takes up its position well towards the apex of the pyramidal gamete. The large nuclear cap, the development of which is now being followed, is still applied to the nucleus, but it is external to the nucleus, and lies nearer the wall of the gametangium. Its bulk is pressed



FIGS. 31-33. 31. Insert from a female gametangium. A chondriosomal complex of large granules drawn about the individual nuclei. Gilson, iron-alum haematoxylin. $\times 1,200$. 32. Female gametangium showing the orientation of gametes. Also orientation of nucleus and nuclear cap in individual gametes. $\times 1,200$. 33. Monster with three nuclear caps fused, three nuclei free, and three cilia. From living material. $\times 1,200$. C. = chondriosomes; N. = nucleus; No. = nucleolus. N.C. = nuclear cap.

into the broadened end of the pyramidal gamete, and takes on the appearance of a heavy crescent-shaped nuclear cap. Apparently, by the crowding of the gametes, the bulky nuclear cap has been forced to develop asymmetrically in its later stages so that the nuclear cap takes up its final position to one side of the nucleus, that side where space permits. That a very considerable pressure is exerted on these gametes by their crowded position is evident from the fact that the nucleus and its attendant cap when first formed take the form of their mould; they become definitely pyramidal. Although the nucleus is not completely enveloped by the nuclear cap it is deeply embedded in it.

Most of the lipid granules in the delimited gametes line up along the cleavage furrows, but there are always some that hug the nucleus and nuclear cap closely. Ultimately the single cilium appears. The cilia of all the peripheral gametes develop at the pointed or inner end of the gamete, and extend into the central region of the gametangium, where there is usually some free space after cleavage has occurred (Fig. 32).

In the formation of this nuclear cap chondriosomes alone are apparently involved. In the living gametangia the lipid granules can be followed

through gametogenesis and into the gametes. Furthermore, during cleavage when the nuclear cap is being formed toward the centre of the gamete the lipid granules are concentrated peripherally (Fig. 7). In applying an osmic acid technique to stain the lipid granules it can be even more convincingly demonstrated that these granules are thrown out into the peripheral cytoplasm, while the nuclear cap is being organized toward the centre of the gametes.

The gametes (Fig. 30).

The study of fixed and stained gametes is instructive in demonstrating the separate identity of the two closely associated structures, nucleus and nuclear cap. For example, the Feulgen reaction for chromatin differentiates clearly between these two structures. While the nucleus proper gives the characteristic pink-to-red reaction the nuclear cap does not stain, but stands out a clear white (Fig. 20). Another critical coloration is that obtained by a combination of Benda fixation and Champy-Kull staining. With this chondriosomal technique, the nuclear cap stains blue, the nucleus red. Even with iron-alum-haematoxylin after either Gilson or Feulgen fixation a clear distinction can be seen between nucleus and nuclear cap.

Observations on the motor apparatus as made on fixed gametes have been incorporated in the description of living gametes.

V. DISCUSSION.

Sex segregation.

In a sexual mycelium each nucleus in the mycelium must be like every other nucleus in its genetic constitution, since all are descendants of the same nucleus, namely, the single nucleus present in the zoospore from which the mycelium arose. From this it follows that in the nuclei of the male and female gametangia at the time when they are delimited from the hypha there can be no difference in genetic constitution. Furthermore, since the nuclei of the hyphae are segregated to male and female gametangia on a random basis the nuclear number is approximately equal (Fig. 16). If then the complement of nuclei in the two gametangia is the same qualitatively and quantitatively at the time when they are delimited from the hypha, when and how do the differences arise that determine that one structure will be male and the other female? Obviously these differences should be traceable to some protoplasmic constituent of the hyphal tip, and obviously to some protoplasmic inclusion other than nuclei. As has been noted before, there are at least two types of clearly defined cytoplasmic inclusions within the hypha: the lipid granules and the chondriosomes. Since the lipid granules are rather evenly distributed through the hyphal tip, and are segregated in male and

female gametangia like the nuclei, i.e. in approximately equal numbers, it seems highly improbable that they play any important role in sex segregation. The chondriosomes, however, are unequally distributed through the hyphal tip. In the tip they are crowded (Fig. 1), but are much more diffusely distributed behind. As the cross walls that delimit the gametangia are formed approximately simultaneously, unequal distribution of chondriosomes between the terminal female cell and the subterminal male is likely to result. It is, however, altogether too early to conclude that the chondriosomes are the certain and exclusive agents of sex segregation in *A. arbuscula*.

Sex expression.

In the expression of sexuality, however, the role of the chondriosomes is certainly of primary significance. Between male and female gametangia a quantitative chondriosomal difference is apparent. Between male and female gametes the disparity in amount of chondriosomal material is still more apparent. The chondriosomal nuclear cap of the female is always three to four times as large as that of the male (Figs. 8 and 30). In the chondriosomes, then, we have a definite quantitative index of sexuality, i.e. of maleness and femaleness.

Another distinction between the sexes in *A. arbuscula*, quite as remarkable as this differential distribution of chondriosomes, is the difference in colour between male and female gametangia and male and female gametes. These distinctions depend on the different colours of the lipid granules in the two cells. Since the brilliant male colouring does not develop until rather late in gametogenesis when the male gamete origins have become rather well organized and the maleness of the gametangium has become relatively well established, it would seem that these cytoplasmic inclusions play no part in sex segregation. Their ultimate pigmentation is, however, a striking qualitative expression of maleness, their lack of pigmentation, of femaleness.

The size of nuclei in gametangium (Fig. 5) and gamete (Fig. 8) is another index of maleness and femaleness in that the male nuclei are but half the size of the female nuclei. This contradistinction, however, seems almost certainly to be the expression of a nuclear-plasma ratio than an expression of sexuality itself. The direct expression of sexuality in this particular is the differential mitotic potentialities developed in the two gametangia. The nuclei were segregated to male and female gametangia in approximately equal numbers. Why the nuclei in a smaller cell should later undergo roughly twice as many mitoses as the nuclei in a larger cell is difficult to explain. The most likely explanation is that there is a fundamental difference in the cytoplasm of male and female gametangia, which difference is capable of affecting the division rate of the nuclei.

The nuclear cap.

Ever since 1878 when Reinsch (14) first discovered *Blastocladia*, students of the water moulds have been puzzled by the peculiar type of nucleus found in the zoospores of this genus. Since 1911 when Butler (3) discovered *A. arbuscula*, other investigators have been interested in the nuclei of the zoospores of *Allomyces*, because these show the same peculiarities as those of the zoospores of *Blastocladia*. Despite the interest shown in these nuclei, no adequate explanation of their singular structure has yet been given; nor has there been any consistent progress in our knowledge of their structure. Barrett (2) in 1912 interpreted these nuclei correctly, concluding that the major portion of the 'large, subtriangular, centrally located body' in the zoospores of *A. arbuscula* (his *Blastocladia strangulata*) was a 'food body', the nucleus being simply imbedded in this 'food body'. Kniep (11) 1929 accepted Barrett's interpretation for *A. javanicus*, but Cotner (6) 1930, working on Thaxter's *B. pringsheimii*, turns back to Thaxter's (16) original misconception that the 'very large and subtriangular' body was all nucleus.

Although the present paper treats of gametes rather than of zoospores it nevertheless presents evidence bearing on this problem of the organization of the 'subtriangular' body in the zoospores of the Blastocladiaceae. In *A. arbuscula* gamete and zoospore are alike in possessing a nuclear cap, which has been shown to be extranuclear. It has been demonstrated, further, that this extranuclear body or cap is formed from chondriosomes. It can, therefore, be stated with confidence that Barrett's interpretation of the nuclear cap (his 'food body') was certainly correct for the zoospores of *A. arbuscula* and, judging from Thaxter's and Cotner's descriptions and figures, it seems certain that the same explanation holds for the 'very large subtriangular' body described by them in the zoospores of *Blastocladia pringsheimii*.

The discovery of a formed body of chondriosomal origin (the nuclear cap) (Fig. 8, 20, 29, 30, 32, and 33) in the gametes and zoospores of *A. arbuscula* is interesting in a limited sense because it explains the structure of certain anomalous nuclei in the zoospores of the Blastocladiaceae. It is interesting in a larger sense because it is apparently the first time that such a structure has been described in the motile cells of plants.

Although a search of the literature on zoosporogenesis, gametogenesis, and spermatogenesis reveals no strictly analogous structure there is in the spermatozooids of mosses a bulky cytoplasmic inclusion that may prove to be the equivalent of this nuclear cap. This structure, in the mosses is known as a limosphere. The term limosphere was first used by M. Wilson (19) in 1911 in describing a cytoplasmic inclusion that he had noted in the spermatids of certain mosses. Allen (1) in his investigation of spermatogenesis

in *Polytrichum juniperinum* was the first to make a careful study of this structure. The limosphere, according to Allen, first makes its appearance in the young spermatid (androcyte) as a spherical, evenly staining mass. This gives off an apical body which becomes applied to the anterior end of the nucleus. The major part, or limosphere proper, temporarily becomes lodged against the posterior end of the nucleus. Later the limosphere remnant disappears from the spermatozoid. The derivation of this limosphere from plastids was first demonstrated by Weier (17) who showed that the 'kinoplasmic plates' of Allen, which appeared in the early spermatogenous cells (androgones) but which disappeared from the androgones before their last mitosis, were actually plastids as had been earlier suggested by Sapehin (15). Weier was not only able to trace the 'kinoplasmic plates' back to the plastids in the young antheridia, but he was able to follow them through the androgonial divisions and into the androcytes where they gave rise to the limosphere. Motte's (13) description of the origin of the limosphere is somewhat different from Weier's. Motte believes that the plastids in the androgonial cells fragment, and then, in the androcyte, join with the chondriosomes already present in the cell to form a granular mass which condenses to form the solid limosphere.

In *Allomyces* it can be definitely shown that the nuclear cap is formed from a complex of chondriosomes. Whether the limosphere of *Polytrichum* is formed from a chondriosomal complex or a plastid complex seems to be only a matter of interpretation. Motte, as we have seen, believes that the limosphere may be formed of chondriosomes, at least in part, and Weier's figures of his plastid complex, as he traces it through the androgonial divisions and into the androcyte, certainly suggest that it may be just as well considered as a chondriosomal complex. His 'granular plastids' and his plastids that look like 'a delicate chromatic reticulum embedded in an achromatic matrix' certainly appear more like the accepted picture of a chondriosomal complex. Judging from Weier's figures and my slides, I believe that the limosphere in *Polytrichum* is built up in quite the same manner as the nuclear cap in *Allomyces*; that is, the chondriosomes first aggregate (Fig. 25), then vesiculate (Fig. 27), and finally fuse (Figs. 27-9). In the process of fusion however, they first form a fine reticulum (Fig. 27), later, a heavier reticulum (Fig. 28), and ultimately a single mass (Fig. 29). Thus it would appear that the origin of the limosphere and the nuclear cap, if not exactly the same, is yet very similar.

It has been suggested (Weier, 17) that there is a close similarity between the limosphere of *Polytrichum* and the acroblast of certain animal sperms. In the case of *Allomyces*, however, if any analogy is to be drawn between the nuclear cap in gamete and zoospore and any structure in the animal sperm it apparently must be with the 'nebenkern'. Here the

analogy seems to be very close. In the sperms of certain Diptera, for example, it has been shown that after the last spermatogonial division, as the interzonal fibres are pinched in two, the chondriosomes that were arranged along the sides of the spindle are pinched apart. The chondriosomes are thereby divided roughly into two equal groups and are left clumped in the interzonal region of each spermatocyte. In this chondriosomal aggregate the individual chondriosomes come to lie more or less parallel to each other, and by a process of progressive vesiculation fuse along their long axes to form a single large mass, the nebenkern.

The developmental history of nebenkern and nuclear cap run parallel with each other most remarkably: (1) both structures are formed out of a chondriosomal complex, (2) in both this complex arises from the vesiculation of individual chondriosomes, and (3) in both the structures form a single large mass.

VI. SUMMARY.

1. In the hyphal tips of *A. arbuscula* there are numerous nuclei, lipid granules, and chondriosomes. Nuclei and lipid granules are distributed at random; the chondriosomes are concentrated in the tip. The nuclei have six chromosomes; the lipid granules are grey-black in colour, small and spherical; the chondriosomes are long and filamentous.

2. When the male and female gametangia are cut off from a hyphal tip the nuclei and lipid granules are distributed between the male and female gametangium in approximately equal numbers. The chondriosomes segregate unequally, a disproportionately large number going to the terminal, female gametangium.

3. In early gametogenesis the nuclei divide more often in the male gametangium, so that it contains roughly twice as many nuclei as the female. These male nuclei are one-half the size of the female nuclei. The chromosome count, however, is six in the nuclei of both gametangia. These nuclei become the 'organization centres' in the formation of gametes, and each collects about itself a sheath of lipid granules and a tangled mass of fine, filamentous chondriosomes.

4. In late gametogenesis the lipid granules in the male gametangium change colour, becoming salmon-pink, while those in the female remain grey. The chondriosomes fragment into a cloud of small granules, and these granules subsequently enlarge and fuse to form, first, a reticulate mantle about the nucleus, and finally, a single large mass which becomes appressed to the nucleus in the form of a nuclear cap.

5. The female gamete is two to three times as large as the male; its nucleus is twice, and its nuclear cap three to four times, as large as that of the male. Its lipid granules are grey, while those of the male are of a brassy colour.

6. Since the nuclei in the hyphae are genetically the same and are segregated in male and female gametangia in equal number, there appears to be here a peculiar type of differentiation in which the distribution of chondriosomes is of primary importance in the determination of sex.

7. In the visible expression of sexual differences there is a definite quantitative factor in the amount of chondriosomes (nuclear cap) and a qualitative factor in the colour of the lipoid granules.

8. The extranuclear nature of the nuclear cap found in the gametes and zoospores of *A. arbuscula* is demonstrated and its chondriosomal origin described. An analogy is suggested between this nuclear cap and the limosphere found in the spermatozoids of mosses. In animals the nebkern of certain spermatozoa (Diptera) suggest even closer analogies.

To Dr. Duncan S. Johnson under whose direction this research was carried out, I wish to make acknowledgement of his stimulating and helpful criticism; to Dr. John N. Couch, who from a distance has guided and directed this study, I also wish to make acknowledgement; and to Mrs. Helen R. Rosenfeld, Miss Elizabeth H. Gay and Dr. William L. Doyle, who have all actively interested themselves in this research, I wish to express my gratitude.

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Wound Healing in *Tradescantia fluminensis* Vell.

BY

ROBERT BLOCH.

With eleven Figures in the Text.

CONTENTS.

	PAGE
I. INTRODUCTION	651
II. ANATOMY	652
III. THE FORMATION OF WOUND CORK IN THE INTERNODE	653
A. General	653
B. Special. Effects of Wounding	655
(a) Epidermis and Collenchyma	655
(b) Cortical Parenchyma and the Sclerenchyma Sheath	656
(c) Pith Region	659
(i) The Vascular Bundles	659
(ii) The Pith Parenchyma	663
IV. WOUND HEALING IN THE ROOT	663
V. DISCUSSION	664
A. Wound Reactions	664
B. The Formation of Crystals of Calcium Oxalate after Wounding	666
C. Grafting in Monocotyledons	668
VI. SUMMARY	669
LITERATURE CITED	670

I. INTRODUCTION.

THE processes of wound healing have been described for roots of Monocotyledons (2), and it was thought desirable to make a similar study on a Monocotyledon stem.

A description of the normal anatomy and development of *Tradescantia fluminensis* has been given by Scott and Priestley (11), but the present investigations have necessitated a further study of changes occurring in older internodes of normal shoots. A paper by Krenke (6), dealing with grafting experiments of *T. fluminensis* with *T. zebrina*, was not seen until the present paper was almost completed. The new formations after wounding, to be described in this paper, would appear to have considerable bearing on the fusion processes described by him.

II. ANATOMY.

In the normal stem of *T. fluminensis* developmental study has led to the classification of the bundles into four series, the medullary, perimedullary, cortical, and peripheral. The sheaths of the latter bundles are coalescent with the zone of 'sclerenchyma', which separates the larger celled medulla from the cortex. The differentiation of the latter series of bundles and of the sclerenchyma ring is basipetal (11). In internodes which are still extending, the sclerenchyma is completely undifferentiated. The first signs of lignification were observed in the third fully extended internode, and development was not complete until the sixth, and even at this stage it was more strongly marked at the upper end of the internode. As in the majority of the other Commelinaceae, the fully developed sheath is one to two cells in width.

No other sclerenchymatous elements occur in normal internodes, but deviations from this are sometimes seen in older and less vigorous internodes. As such differences may be due to particular external conditions, it is not safe to draw inferences as to the age of the internodes from them. Such internodes may be especially solid and show considerable variation in the number of lignified elements, which, as in other Commelinaceae, tend to occur sometimes as single cells, either in the pith or in the colourless layer immediately external to the normal sclerenchyma and especially opposite to the bundles. Similar microchemical changes may affect the cells around the cortical or perimedullary bundles, but lignification around the medullary bundles, though described for some Commelinaceae, has not been observed in *T. fluminensis*, and in *T. virginica* only in the rhizome (4).

In *T. fluminensis* thickening and lignification may occasionally occur in the pith between the medullary bundles or, rarely, of all the large pith cells, but the small parenchyma cells around the xylem cavities of the inner bundles never lignify.

The old parts of procumbent stems contain chloroplasts and numerous starch grains, and abundant starch also occurs in the reddish white part of the stem in the soil. In such parts also the sclerenchyma sheath is only appreciably developed outside the phloem and is frequently interrupted by completely unlignified cells.

In *T. fluminensis* it was found possible to induce the formation of the characters of a rhizome in the following manner. Cuttings consisting of about five internodes and taken from the top of plants were set in pots in June 1933. They were cut below a node and planted so that the lowest internode was completely covered by the soil. Roots appeared freely at the node at the top of the buried internode and a few also from the lower node. On investigation of this internode, six months later, it was found to

be whitish in colour, the sclerenchyma sheath was thin-walled and interrupted in places, and abundant starch was present. The cells around the peripheral bundles were sclerenchymatous, the thickenings being

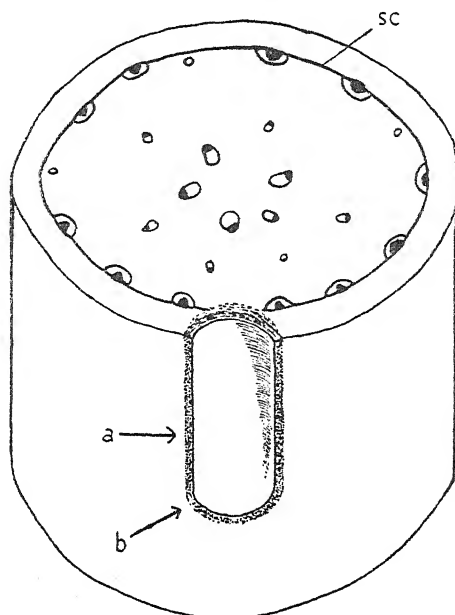


FIG. 1. Diagram showing the structure of the internode and wound cork formation round a wounded part of the cortex. (sc) sclerenchyma sheath.

especially marked outside the phloem and characteristically U-shaped. In contrast to this, the next internode above the soil developed a normal sclerenchyma sheath. The development of the rhizome in *T. virginica* is similar (4, p. 114).

III. THE FORMATION OF WOUND CORK IN THE INTERNODE.

A. General.

The material used for wounding in the summer of 1933 consisted of uniform vertical branches of vigorous growth. The age of the internode was noted, and wounding was effected by cutting away a small portion with a razor (Fig. 1). The plants were examined either a few days or several months later. Immediately on wounding a quantity of mucus was released, and the raphides contained in this persisted a considerable time after wounding. After healing, the wound is surrounded by a small brown rim, which is often somewhat raised owing to meristematic activity beneath it (Fig. 2). Beneath the rim, and varying according to the depth of the wound, a continuous meristem extends through all the living tissues, as

will be described in more detail in the special sections. In examining the meristematic activity care has to be exercised or divisions in certain planes are liable to be overlooked.

The brownish walls of the external part of the wound are suberized.

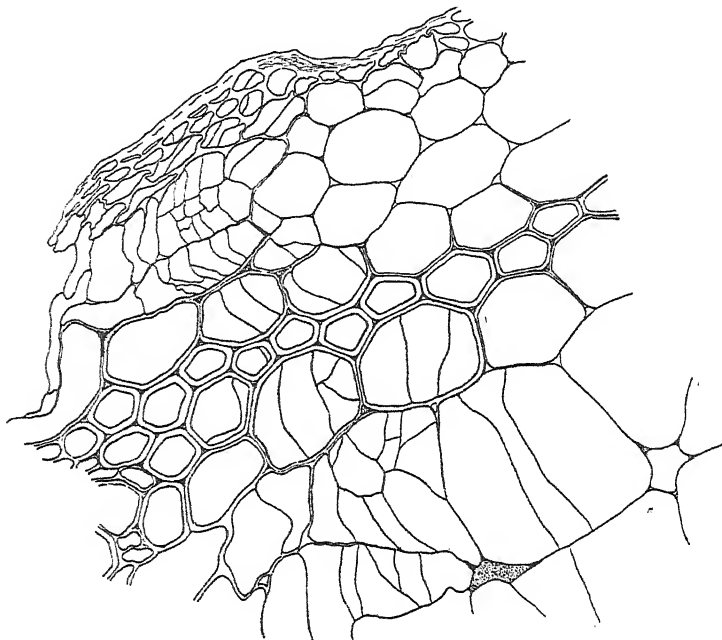


FIG. 2. Transverse section of an internode showing the wound-margin with cell divisions in epidermis, collenchyma, cortical parenchyma, sclerenchyma sheath, and pith parenchyma, and slightly thickened cells in the neighbourhood of the sclerenchyma sheath.

The impregnation is especially strong in the original walls of the collenchyma and sclerenchyma, which also develop suberin lamellas. Dark pigmentation often appears in the cavities of the cells of the wounded region also. The wound cork is of the typical Monocotyledon 'Etagen' type. The radial series of cork cells may, however, appear relatively long owing to approximately radial arrangement of the original cork mother-cells and the difficulty of delimiting these when the small intercellular spaces have become blocked. The external suberized layers consist of cells which divided and then ceased growth. In transverse section they are small rectangular cells, the walls of which are frequently folded. The inner, unsuberized layers of wound tissue are full of protoplasm, contain conspicuous nuclei, and may later develop into typical 'Etagen' cork.

Deep wounding may cause vigorous growth of the parenchyma cells, which extend enormously towards the cut surface. This is especially marked when the cut surfaces are in contact as in split internodes, when, after a few weeks, both halves are almost rounded in cross-section. This

is also observed when a small piece is split away, even when it only contains a few pith cells.

The wound tissues are always free from starch. The big cells of the

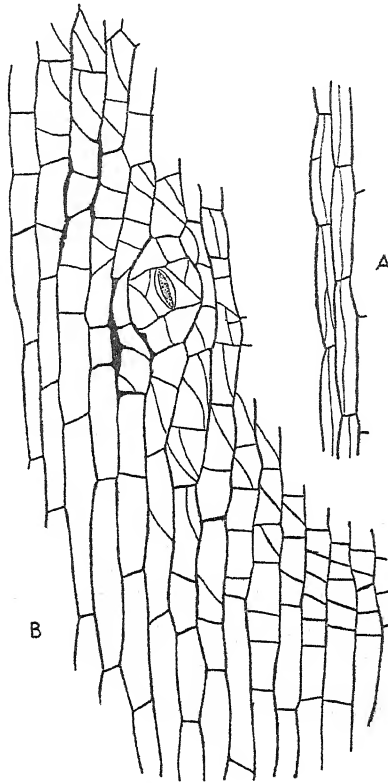


FIG. 3. Surface section to show the distribution of cell divisions in the epidermis. (Cp. the regions marked (a) and (b) of Fig. 1.)

wound parenchyma often contain two or three nuclei, or the nuclei may be lobed or fragmented, as has also been described for *T. virginica*.

B. *Special. Effects of Wounding.*

(a) *Epidermis and Collenchyma.*

The epidermal cells around the wound do not change their form after wounding, but divide several times by new walls which are usually orientated parallel to the wound margin. The guard cells of the stomata collapse and the cells around them divide several times. In the collenchyma the new walls are mainly vertical or oblique. Owing to these various planes of the new division walls, this activity is best studied in sections parallel to the surface (Fig. 3).

The divisions in the epidermal cells are subsequently followed by death of the protoplasts and a change in the microchemical behaviour of the walls. This is evidenced by the brown coloration of the rim of the wound in the fresh condition. After treatment with alcoholic hydrochloric acid such walls give no blue colour with methylene blue, either in the epidermis or collenchyma. After Eau de Javelle for twenty-four hours, methylene blue gives a blue colour. The fatty impregnations of the walls are only removed very slowly from the walls by Eau de Javelle, but after prolonged treatment only a suberin lamella continues to give the fat reaction with Sudan III. The suberin lamella can now be seen to be distinct from the original cellulose wall, and sometimes a thin tertiary cellulose lamella may be seen to have been laid down subsequently within the suberin lamella. Even without meristematic activity, the formation of such suberin lamellas and the strong impregnation of the zone of massive collenchyma cells must provide a very efficient blocking of the wounded tissues.

(b) *Cortical Parenchyma and the Sclerenchyma Sheath.*

Superficial wounding readily causes meristematic activity in the epidermis, collenchyma, and cortical parenchyma. Deeper wounds induce reactions in the sclerenchyma sheath, but this tissue responds less readily than other tissues, and also progressively less the more fully differentiated it becomes. The sheath thus forms to some extent a barrier against the influence of the wound upon the pith tissue. This is comparable with the effect noticed in earlier experiments on roots of Monocotyledons (2), when removal of the cortical tissues as far as the endodermis induced no divisions in the axial strand.

In the present experiments the wounds were kept moist by putting the stems in water, the level of which reached to within a short distance of the wound. Superficial wounding by clean cuts caused reactions in the tissues outside the sheath only, but the slightest injury of the sheath cells themselves was associated with divisions in the pith also. Some methods of wounding, such as tearing off the epidermis, caused injury and collapse of some of the cells of the assimilating parenchyma; some of the neighbouring sclerenchyma cells also collapsed, and divisions occurred in cells of the sheath and also in the region of the pith within the affected region (Figs. 4 and 5). In such cases the activity in the pith may be so vigorous as to burst through the sclerenchyma sheath, remains of which are subsequently seen at the surface of the wound.

All wounds interrupting the sheath induce divisions in the cells of the sheath bordering the wound and in the pith cells beneath. If the cells of the sheath are older and lignified at the time of wounding, they still retain their living contents, but the reaction is of rather a different kind. The

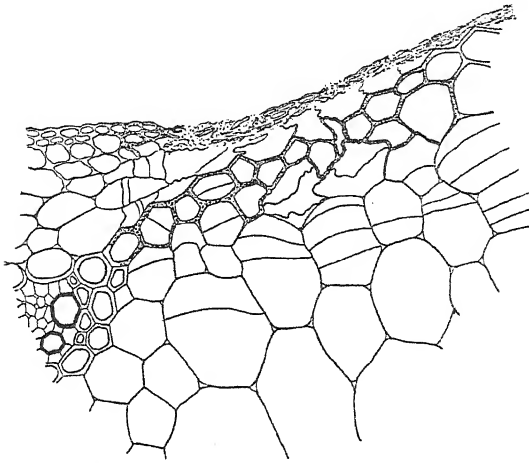


FIG. 4. Transverse section of an internode below node 5, showing meristematic activity in a wounded area caused by scratching. The collapsed dead cells of the sclerenchyma sheath do not contain suberin lamellae.

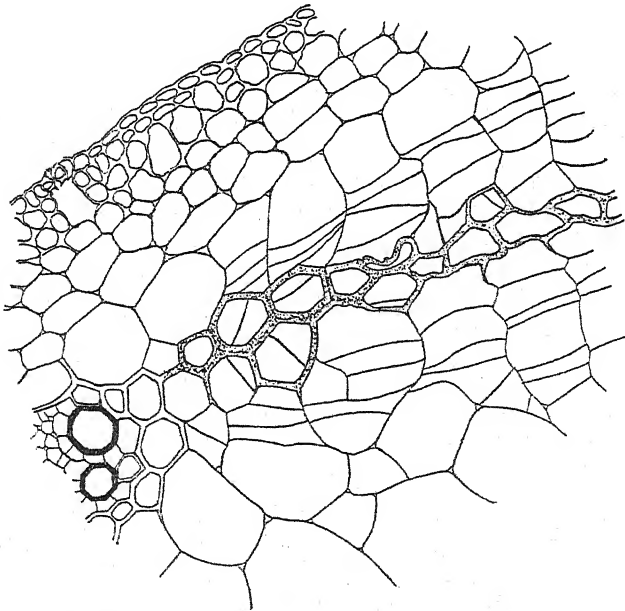


FIG. 5. Transverse section of an internode showing meristematic activity above a wound. The affected part of the sclerenchyma sheath is slightly thickened, yellowish coloured, and does not give the lignin reaction. The collapsed dead cells do not contain suberin lamellae.

walls may thicken, the cells increase in size, or cell-divisions may occur, or sometimes all these three effects may be observed in the same experiment. The wall thickening is sometimes so intense as practically to obliterate the cell lumen. In some cases at the edge of the wound, thickening and

lignification may also affect the neighbouring cells of the pith and of the innermost, colourless layer of the cortex and the products of division derived from them (Fig. 2). All such new sclerotic cells give fat and lignin

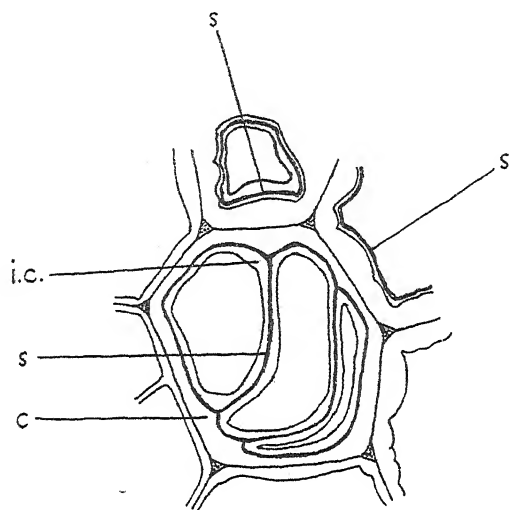


FIG. 6. Small portion of Fig. 2 showing in the centre a parenchyma cell which has become thickened and lignified after wounding. The different layers are seen after 24 hours' treatment with Eau de Javelle: (c) original cellulose wall; (s) suberin lamella; (i.c.) internal lignified cellulose layer.

reactions, but subsequently in some of them death of the protoplasts and a change in the microchemical behaviour may follow. Figs. 4 and 5 show the activity in the neighbourhood of the collapsing sheath cells. In such a region as this the intercellular spaces are generally filled with fatty substances, and internal suberin lamellas are formed in the cells. Single sclerenchyma cells which have divided often become centres around which cell-divisions occur in the pith and cortex. Such appearances suggest that injured sclerenchyma cells may act as necrotic centres, which may in part account for the activity in the cells around. The suberin lamellas show well by staining with Sudan III after twenty-four hours in Eau de Javelle (Fig. 6). The development of an internal cellulose lamella is usual, but it does not appear to be universally present. Thus the original walls show the three layers, lignified primary wall, suberin lamella, and usually an internal lignified cellulose lamella. In the new division walls the suberin lamella appears to be in the middle of the new wall, and cannot be distinguished from a primary lignified region (Fig. 6).

Dedifferentiation and pseudo-regeneration.

Sometimes, instead of the thickening processes described, the sclerenchyma cells lying near the injury and in a region of vigorous meristematic activity become thin-walled, lose their fatty impregnation, and collapse and

disappear in the wound tissue (Fig. 7). Such effects are not seen when the walls at the time of wounding are old and thick-walled, but in younger internodes the characteristics of the sclerenchyma sheath may fail to

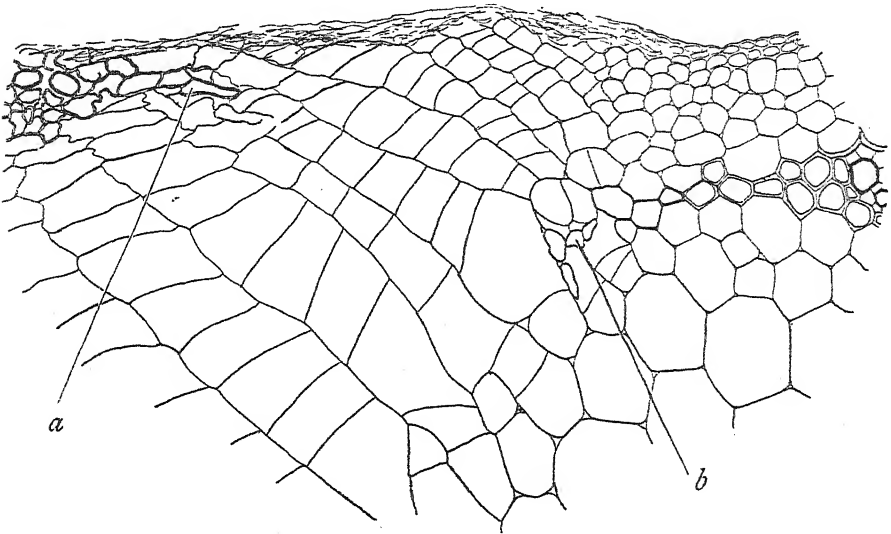


FIG. 7. Appearance of the sclerenchyma sheath in a region of vigorous meristematic activity in a young internode; (a) remains of the original sclerenchyma sheath at the time of wounding, pushed out by the activity of the pith, still showing the fatty impregnation; (b) thin-walled sclerenchyma cells bordering on the wound meristem.

develop in such regions. Such appearances of dedifferentiation may be comparable with the resorption of cells which has sometimes been described in the zone of fusion of grafted plants.

Although direct regeneration of the sheath from other tissues was not seen, an effective replacement of this by tissues of a rather different kind was observed in some cases in a manner comparable with the pseudo-regeneration of the exodermis in the air root of *Philodendron Glaziovii* (2). In some young internodes with superficial wounding, the original sheath had been pushed out by the activity of the pith cells, and in the new tissue so formed cork had differentiated. The original torn edges of the sheath were now connected by cells, which were lignified but less regularly arranged and with a wider lumen than in the normal region of the sheath. Such cells originated from the pith, but developed walls more strongly thickened and lignified than is normally the case.

(c) *Pith Region.*

(i) *The vascular bundles.*

When wounds extend to the pith region, the pith parenchyma and the living cells of the bundle sheaths respond vigorously. Sometimes a small

degree of response is also seen in the small cells which surround the xylem cavities.

The peripheral bundles, when fully differentiated, are enclosed in

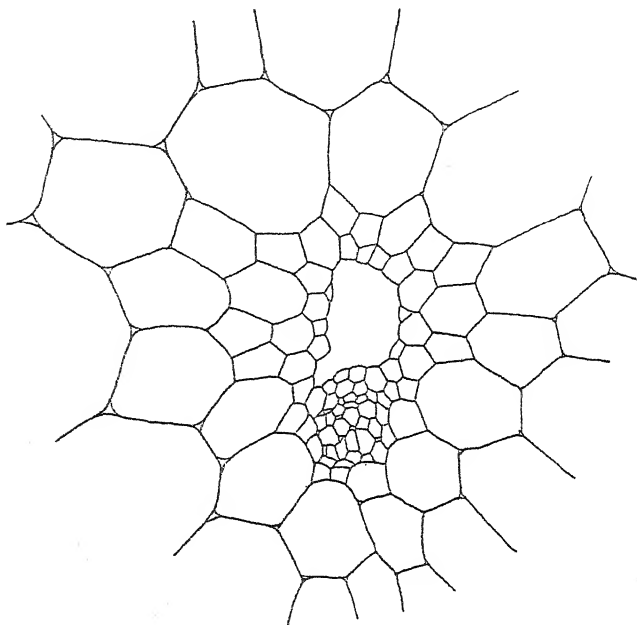


FIG. 8. Transverse section of a medullary bundle at considerable distance from the wound.

a lignified sheath which adjoins the sclerenchyma sheath of the stem. The cells of such a lignified bundle sheath do not react unless directly injured. According to Schubert (10) the sheaths of the pith bundles react more readily at the base of the internode, and may give rise to adventitious roots in cuttings; in the present experiments they reacted in the older, upper regions also.

The meristematic activity gives rise to strands of cells, which run from the pith bundles towards the wound. The xylem cavities may be completely occluded by the proliferation of the surrounding cells, and thus the original positions of the bundles may eventually be indicated merely by the presence of narrow strands of cells.

In the case of a mature (eighth) internode wounded on July 14, 1933, and examined two weeks later, some of the sheath cells of the medullary bundles at a considerable distance from the wound surface had become thickened by a secondary lignified and fat-impregnated layer, which was also conspicuously pitted. The impregnation appeared first in the region of the wall abutting on the cell lumen. Nearer to the wound all cells of the bundle sheath were strongly thickened and lignified. In very deep

parts of a wound, the meristem extended to the medullary bundles, and here the cells of the bundle sheath and those around the xylem cavities contributed to the meristem (Fig. 9). On the other side of the bundle

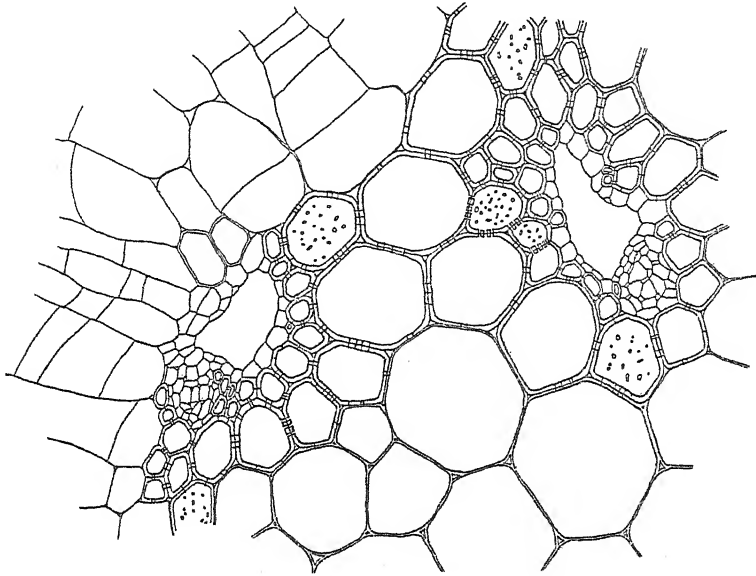


FIG. 9. The same bundle, immediately bordering on the wound meristem, connected up with another bundle by thickened and lignified pith cells.

certain of the sheath cells became thickened and lignified. Thickening and lignification of pith cells also occurred, and thus the bundles became connected up on the side towards the wound by a zone of thick-walled cells. A few cells of the phloem may also sclerify. Two different stages in the same bundle at different levels are shown in Figs. 8 and 9 (left). Doubtless all cells, which in thin, old internodes, may give the lignin reaction (see p. 652), are capable of sclerifying in wounded internodes. Fig. 10 shows a case of vigorous reaction in the sheath cells of two medullary bundles in an internode wounded on September 12, 1933, by an oblique cut, and examined two months later. Thick-walled and lignified cells were produced by the parenchymatous sheath cells and by some of the small cells around the xylem cavities. These observations show how vigorous is the potential activity of the living sheath cells of the medullary bundles even after the xylem has been ruptured. Such cells are able to develop practically the same microchemical properties as those of the normal sclerenchyma ring of the stem. The impregnation is, however, rather more readily removed, and the violet pectin reaction is given with methylene blue after treatment with Eau de Javelle, whilst the cells of the normal

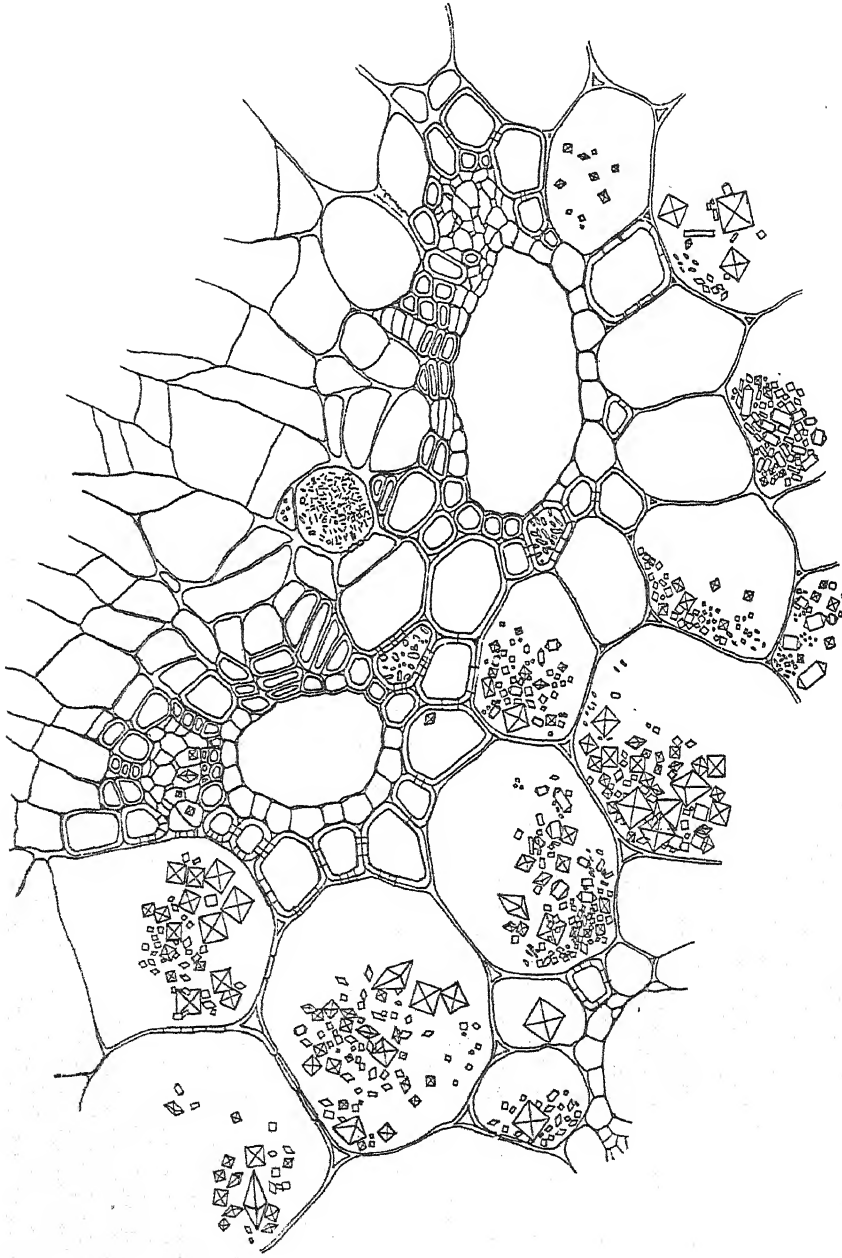


FIG. 10. Transverse section through the region of two medullary bundles two months after wounding, showing both meristematic activity of the sheath cells and formation of thickened and lignified cells. Note the deposit of calcium oxalate crystals in the adjacent pith parenchyma.

parenchyma ring still give the blue lignin reaction even after treatment with Eau de Javelle for four days.

In the normal unwounded cells of the bundle sheaths the contents are richer in protoplasm and contain more numerous granules than those of the surrounding pith cells, and apparently this, combined with the increased metabolism due to wounding, is sufficient to induce a further differentiation of the sheath cells.

(ii) *The Pith Parenchyma.*

In the general ground tissue of the pith, the first observed effect of wounding is the blocking of the intercellular spaces with substances of a fatty nature. Following this the cells towards the injury divide; farther away from the wound cells may thicken their walls, often without change in external form, and both the original walls and the cells derived from them by division may contribute to a cell complex of thick-walled, lignified, and conspicuously pitted cells (Fig. 9).

In limited areas in the neighbourhood of a wound sometimes the blocking effect may be so intense as to affect, in addition to the intercellular spaces, the cell lumen of some of the cells which become filled with brownish contents. Such cells often are surrounded by others which are dividing. The processes observed resemble those occurring nearer to the injury in the sclerenchyma sheath (see p. 658). The effects of blocking of the spaces and the production of gum-like masses which occupy the former position of intercellular spaces is seen in Fig. 11. (In some preparations clear indication is also seen that the wall thickening is due to the deposition of internal layers.) The walls in this region do not give lignin reactions but stain with Sudan III, and often have a definite suberin lamella. According to the literature, the changes in the neighbourhood of a wound may consist of cell divisions in the cells around blocked air-spaces, increase in cell size, or increase in wall thickness. In the present investigation several of these phenomena occur simultaneously, but so far it has not been possible to analyse them. It would be of interest to investigate further the cases where accompanying increase of wall thickness is not reported, as, for example, in the injection experiments of Reiche (9). In *Tradescantia* all cells tend to resume wall thickening as the result of wounding, so it would not be a suitable plant for such an analysis of the wound effects.

IV. WOUND HEALING IN THE ROOT.

The reaction of the root tissues of *T. fluminensis* corresponds to that of the stem, and is similar to that of roots of other Monocotyledons observed in earlier experiments (2). Some stems were cultivated in tap water, and young adventitious roots were wounded on July 4, 1933, by cutting out lateral segments with a razor. They were investigated six

days later. The roots at the level of the wound possessed long root hairs, and a suberized exodermis. Where the cortex had been wounded, exodermis and cortical parenchyma formed wound cork (Etagen kork). If

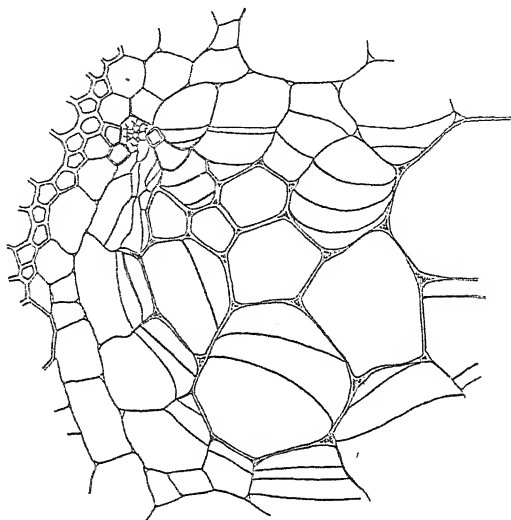


FIG. 11. Transverse section of an internode showing an area consisting of thickened pith parenchyma cells surrounded by cell divisions in the neighbourhood of a wound caused by pricking. The intercellular spaces are blocked, and suberin lamellae are formed.

only the exodermis was wounded, the next layers within adopted its structure, and thus a compensation took place. Sometimes all cells of the cortical tissue became suberized as far as the endodermis and, on the affected side of the axial strand, the endodermis had stronger U-thickenings than on the other sides. Here also the endodermis acted as a barrier against the effects of wounding: but if the endodermis itself was wounded cell divisions occurred in the axial strand. Wound cork formation may take place in all living cells of the axial strand. The parenchyma and the cells of the pericycle divide very readily, and become elongated towards the cut surface, whilst the vessels become blocked by tylosis, and the external cells are suberized.

V. DISCUSSION.

A. Wound Reactions.

Wound reactions occur in living cells of all wounded tissues, varying in degree according to the proximity to the wound. In the internode a continuous meristem is formed through epidermis, collenchyma, cortical parenchyma, sclerenchyma sheath, and pith parenchyma. The reactions may consist of cell divisions, cell elongation, and wall thickening. In the

external part of the wounded region a blocking of the intercellular spaces takes place, and also the formation of suberin lamellas and the impregnation of the original walls of the cells. Simultaneously with these reactions degenerative processes may occur, and also localized meristematic activity may often be seen occurring around sclerenchyma cells which act as a centre. Products released from the cells affected by the injury may be transported a certain distance through the intercellular space system into the subjacent parenchyma, where they may influence the vital processes of the cells in a similar manner to those in the wounded region itself. Both degenerative processes and meristematic activity occur in this region. The changes in the metabolism after wounding and the different distribution of the nutrient material, both of which are closely connected with the formation of the meristem, are thus not confined to the wounded tissues themselves, but extend over to the unwounded region. Noteworthy effects of this are the decrease of carbohydrates, increase in protein, and the accumulation of oxalic acid ('wound oxalate') (cp. p. 666). In the region adjacent to the wound tissue, cells generally tend to resume development and undergo a thickening of the walls, together with a deposit of substances giving fat and lignin reactions. Similar reactions to this are also characteristic of thin old internodes, and thus should be regarded as somewhat distinct from those chemical changes which take place in the external zone of the wound tissue. Development can be resumed in all living, vacuolated cells of the older tissues, which also easily form wound cork, thus showing their high capacity for renewed meristematic activity or for acceleration of developmental processes which are normally very slow. This resumed development is more vigorous in those zones which are richer in protoplasm and have remained more or less potentially meristematic, as, for example, the sheath cells of the internal bundles or the cell layers lying immediately to the outside or inside of the sclerenchyma sheath. If wounded, these cells easily reach a sclerenchymatous stage, which is unknown in the normal internode, and they may also divide. Such secondarily sclerified cells adjacent to the wound tissue are found to differ in the degree of lignification from the cells of the original, external sclerenchyma sheath as the impregnating substances are more easily removed by treatment with Eau de Javelle.

The thickening of cell walls in the neighbourhood of wounds is characteristic of wound healing in Monocotyledons as also in other plants. According to Holden (5), similar changes occur in petioles and stems of ferns. Very probably these changes are a consequence of active metabolism in the wounded organ, especially effective in potentially meristematic zones. Thus in the roots of Monocotyledons both endodermis and pericycle easily become thickened (2) since these layers are relatively late in becoming differentiated, as is shown by the production of branch roots from

the pericycle. Similarly in *T. fluminensis* the internal bundle sheaths and the zone of cells outside the external sclerenchyma sheath are potentially meristematic, and may give rise to adventitious roots at the base of cuttings (10). The investigation of thin old internodes in conjunction with the wounding experiments have shown that the tendency to sclerify is not confined to the cells of the original sclerenchyma sheath, but lignified and thickened cells may also occur in the medullary region. The external sclerenchyma cells are, however, characterized by their early development, great length, and small lumen. Such cells do not arise from a cambium, but are formed by cell divisions of the fundamental parenchyma, and the cross walls are straight. They contain neither starch nor crystals. The external sclerenchyma sheath, if already differentiated at the time of wounding, generally continues development by thickening the walls, and responds less readily than other tissues by cell divisions. It is a feature of this layer also that it forms a barrier to the spread of the wound effects to the tissues within, even when it is at a relatively early stage of development.

B. The Formation of Crystals of Calcium Oxalate after Wounding.

In unwounded internodes and in the leaf of *T. fluminensis* a few solitary crystals of the tetragonal calcium oxalate trihydrate may occur as well as the normal content of monoclinic raphides. Investigation of wounded internodes, however, showed the presence of numerous solitary crystals, the greatest number occurring in the pith parenchyma in the immediate neighbourhood of the wound-meristem (Fig. 10). Deep wounding causes a much greater accumulation of solitary crystals than superficial wounding.

The formation of the solitary crystals is completely isolated from the normal formation of the raphides. After the first period of raphide formation in the plant further calcium oxalate becomes deposited in the form of solitary crystals. After wounding of the internode the raphides do not change. The forms of the solitary crystals produced after wounding are identical with those normally occurring. Frequently bipyramids were found, or the combinations of pyramid and prism of a very different length. The size of the crystals varied. If only a small amount of calcium oxalate was formed after wounding, big bipyramids were generally found. If there was a larger deposit, often very different forms and habits occurred together in one cell. Bipyramids with a side $21\ \mu$ long in optical section, and rod-like crystals with a prism $45\ \mu$ long, and also very small crystals occur, the crystal form of which cannot be recognized. The crystals do not occur in the meristematic cells of the wound tissue itself, but in the parenchyma cells of the adjacent pith tissue; the sheath cells of the internal bundles and cells which have sclerosed after wounding may also contain crystals.

The circumstances which lead to the formation of wound-oxalate are probably very different from those associated with the raphides. Whilst in the regular formation of raphides the plant itself plays an active part, the formation and distribution of the wound-oxalate presumably depends on the metabolic processes in the region affected by wounding. Regarding the possible character of these processes, an approximate conclusion can be drawn by the comparison of the conditions leading normally to the formation of calcium oxalate, and the chemical changes occurring in the wounded region. Many former observations have shown, in the first place in the leaves of green plants, that the formation of calcium oxalate or of soluble oxalates is often associated with the formation of protein from nitrate. In wounded organs of plants also a considerable increase of protein occurs. According to Friedrich's observations (3) this increase is high in organs which are rich in carbohydrates, and is often associated with an accumulation of organic acids. These changes were stated to occur mainly in storage organs, and to be connected with a high capacity for regeneration. In the green internode of *Tradescantia* similar circumstances may be present. The formation of the meristem and the renewed vitality in the wounded internodes are apparently connected with a large accumulation of oxalic acid. Numerous experiments of former investigators have shown that in plants containing calcium oxalate, the oxalic acid easily becomes deposited if sufficient calcium is available. By the reduction of the calcium nitrate supply or by supplying the nitrogen as an ammonium salt, a plant, normally containing oxalate could be made to produce little or no oxalate. Raphides apparently only need a very small amount of calcium, and it is very difficult to influence their formation. On the other hand, when a large supply of calcium carbonate was given to plants normally poor in calcium oxalate, a large formation of calcium oxalate took place, generally in the form of solitary crystals or some cluster crystals. Plants which store calcium as calcium carbonate, or which excrete calcium, do not behave in this way.

T. fluminensis, like other Commelinaceae rich in calcium, is especially suitable for experiments, and has already been used by Schimper. Benecke (1) has shown that the crystal content of the leaves can be largely influenced by varying the conditions of food-supply as previously discussed. Similar behaviour was noticed by Müller (8) in the leaves of *Callisia repens* and other plants. According to Stahl (12) even isolated fragments of leaves of *T. fluminensis* easily formed 'adventitious crystals' when placed in solutions containing calcium compounds.

Presumably the deposit of calcium oxalate produced after wounding in *T. fluminensis* will not remain an isolated case. In this respect a former observation of Lohse (7) is of some interest. He found large solitary crystals in decapitated stems of *Hippuris*. Applying this observation to

T. fluminensis, it was found in the present experiments that calcium oxalate accumulated in the stumps, when plants several weeks old were decapitated close to the soil level. The accumulation of calcium oxalate in the stump, but not in the subterranean parts, was noticed eight months later.

C. Grafting in Monocotyledons.

The behaviour of the internal bundles and bundle-sheaths is of special interest with regard to the question of grafting in Monocotyledons. All such experiments have hitherto failed, as in no single case was a differentiation of vascular strands between the grafted symbionts obtained. Recently an account has been published regarding investigations of Krenke and Dubrowitzkaja (6, pp. 434-54; Figs. 124-42) concerning the development of xylem and phloem strands between *T. fluminensis* Vell. and *T. zebrina* Hort. The chief results are: the formation either of separate xylem or phloem anastomoses or both arising together from the neighbouring parenchyma of the internal bundles or from the peripheral, latently meristematic ring. There was only a small callus formation between the grafted plants, but meristematic activity took place in the region of the internal bundles, and often strands of cells ran from the internal bundles towards the graft union, in a similar way to that described above as occurring after simple wounding of an internode.

Unfortunately, in the preliminary account of these investigations in the German edition of Krenke's manual it is not indicated which zones of the grafted plants have reacted with each other. This is, however, of great importance as structure of node and internode are not identical (cf. (11) and (4)). Furthermore, the regenerating zone of the Monocotyledons is mainly confined to the nodes; in *T. fluminensis*, as mentioned above, a small zone in the base of the internode may also regenerate (10). According to the figures, in cleft grafting an internodal wedge of *T. fluminensis* has reacted with nodal tissue of *T. zebrina*. It follows, however, that we have to deal in the stock (node of *T. zebrina*) with the special distribution of the bundles normally present from the beginning in the node (cf. Gravis, Pl. XI, XII; Scott-Priestley, Figs. 5a, 5b). The elements described in detail as new formations (pp. 437 etc.), for example—conducting elements outside the peripheral bundles, leptocentric bundles in the central region, connecting strands, &c.—are typical of a normal node, and owing to this original, irregular arrangement of the conducting elements in the node, the proof of new formation of conducting elements due to grafting must become extremely difficult. If there are phloem or xylem elements directed towards the graft union, which may occur very easily, it will be necessary to investigate whether this is due to a 'revival of the activity of the tissue' or a consequence of the arrangement of the conducting elements in the node. The scion (internode of *T. fluminensis*)

gave only an extraordinarily small reaction, as is shown by the figures, and far below the degree of meristematic activity obtained after wounding in the present experiments.

Nevertheless Krenke thinks that in his experiments a real fusion of scion and stock was obtained, in such a way that a change of parenchyma cells outside the bundles into conducting elements had taken place in the scion, and formation of phloem and xylem strands between bundles of the graft symbionts was stated to occur. There are two facts, however, which need consideration in deciding whether this was a real connexion between scion and stock. The first is the destruction of the xylem which constantly takes place in the internal bundles of the scion, the second that only irregularly 'incrusted' thick-walled cells rather than tracheids generally represent the union of xylem of scion and stock. Krenke also refers to the phenomenon of the destruction of the xylem in the central bundles of the scion, calling it obliteration, and suggests that a fusion of the xylem develops more easily from peripheral bundles where no obliteration has taken place. He suggests further that the destruction of the xylem in the central bundles may account for the small degree to which the scion participates in the fusion process (described on p. 668 in this paper). The disappearance of the xylem in the internal bundles is not surprising considering that the rupture of the xylem is characteristic in a normal internode. Regarding the irregularly thickened cells which often represent the union between xylem of scion and stock, it seems that these cells correspond to a remarkable extent to the sclerenchyma cells with reticulate thickenings, produced from the parenchymatous sheath-cells of the internal bundles after wounding as described above. Further observations are required to prove definitely whether these cells have any conducting function, whilst further inquiries regarding the reaction of the node towards wounding would also be of interest.

VI. SUMMARY.

1. The anatomy of certain features of old and thin internodes of *T. fluminensis* Vell. is described for comparison with normal and wounded internodes. The wound reactions of the stem are described in general and also in detail for the various tissues of the stem. These reactions consist of cell divisions, cell elongation, wall thickening, the formation of suberin lamellas and the impregnation of walls with fatty and lignin substances. In some cases processes of dedifferentiation have also been observed.

2. The reactions to wounding are described for the root.

3. The wound reactions are discussed in relation to the redistribution of nutrient materials in the stem and the altered metabolism induced by wounding. One effect of wounding is the formation of unusual amounts

of calcium oxalate crystals, and this is discussed in the light of our knowledge of the distribution and formation of deposits of calcium oxalate. The bearing of the observations on wound healing is discussed in relation to recent experiments on grafting in Monocotyledons.

I wish to express my sincere thanks to Professor Priestley for providing me with the opportunities for carrying out this investigation and for his continued interest in the work, and also to Miss L. I. Scott and Miss E. Smithson for their help in the preparation of this paper.

I would also like to take this opportunity of thanking most sincerely those kind friends who made possible my working at the University of Leeds.

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The Genetics of *Primula sinensis*.

IV. Indications as to the Ontogenetic Relationship of Leaf and Inflorescence.

BY

EDGAR ANDERSON AND DOROTHEA DE WINTON.

With Plates XIII and XIV and ten Figures in the Text.

ONE of the chief methods of scientific experiment, highly desirable but rarely attained, is to vary a single factor at a time, keeping all others constant. The numerous Mendelian recessives isolated and analysed by geneticists provide material which, from this point of view, is of primary importance for experimental work in physiology and morphology. These Mendelian recessives have been demonstrated to differ from their normal allelomorphs by a unit change in their germ plasm. Whatever the nature of this change, there can be no reasonable doubt that it is a unit change, affecting some particular function of the nucleus and that it characterizes all the nuclei of the recessive individuals. In families segregating for one of these unit factors (A and a, for example) we can arrange the progeny in two groups, or at most in three, if the character in question is not completely dominant. In one group will go all those individuals which have received the recessive gene from each parent (the aa's). In the other will go all those which have at least one dominant gene (the Aa's and the AA's). Growing these two groups, side by side, each will have the same range of environmental factors. Other germinal factors will undoubtedly be segregating and will produce differentiation in the experimental material. But if the two groups have been separated on the basis of one set of allelomorphs, the range of germinal differentiation will be the same in both, and the only essential difference between the two will be a unit difference, affecting the germ plasm at a particular point in a particular way.

For an experimental study of morphology in the flowering plants, the genes affecting leaf and flower shape in *Primula sinensis* seem particularly useful. *P. sinensis* is a complex, well-differentiated plant. Its leaves have distinct petioles, its inflorescence is differentiated into peduncle, pedicels, bracts, calyx, corolla, &c. All of these parts of the plants are large and are easily studied by the naked eye, whereas homologous structures in many other genetically analysed species would require microscopical examination.

Furthermore in *P. sinensis* there is available for experimental study an unusually large number of recessive genes having a clear-cut effect upon leaf shape and flower shape. The following paper is an attempt to analyse the effect of ten of these genes, alone and in combination, upon the various recognizable morphological categories in *P. sinensis*, to wit; petiole, leaf blade, peduncle, pedicel, bract, sepal, and petal. From such an analysis one may hope on the one hand to gain a clearer insight into the physiological (that is the ontogenetical) relationships between these categories, and on the other to learn a little something of the way in which particular genes produce their characteristic effects.

METHODS.

For the most part this study has been confined to gross morphology. While detailed anatomical investigation of the material would be highly desirable it has not as yet been undertaken, for two reasons. In the first place, painstaking and scrupulous examination of the gross morphology alone yields significant data. In the second place, it is necessary to establish the main facts in the case before beginning a detailed analysis. Before attributing a particular effect to a particular gene it was necessary to scrutinize a very large number of plants. The genetical nature of the experimental material increased these difficulties, since in addition to the ten genes which had been chosen for special study many other genes were segregating in the families under observation. Therefore a very large number of plants had to be examined in order to establish the various effects of a particular gene. While this could be accomplished if the examination was limited to the gross morphology, it would not have been practicable to have extended the examination to anatomical details.

In 1930, when the experiment was initiated, eight mutant genes were available for study. This meant that if each was to be studied singly and in all possible combinations with the other seven that it would be necessary to synthesize, grow, examine, and record 256 different genetic types. After the work was under way two other useful mutants arose, raising the number of possible combinations to 1,024. A systematic attempt has been made to create and study as many of these combinations as possible. Attention has been turned particularly to the single, double, and triple mutants, since the more multiple mutants are less viable and are difficult to cultivate in large numbers. A standardized photographic record was devised in the first year of the experiment and has been adhered to ever since. Photographs of the corolla, calyx, and bract were made at various magnifications but were uniformly printed (this usually required slight enlargement) at a scale of two diameters. Photographs of the leaves were taken at various magnifications but were all printed on a scale of one-half

diameter. The flower and leaf photographs were mounted on opposite sides of stiff four by six index cards. The projecting 'tabs' of the index cards were used for indexing the various genetic combinations. One pair of allelomorphs (**Ch** vs. **ch**, see below under **sinensis-stellata**) have such a marked effect upon the inflorescence that they are of outstanding importance. The photographs of all plants called '**sinensis**'¹ by primula fanciers (**ChCh** or **Chch**) were mounted on cards with index tabs at the extreme left. Photographs of '**stellata**' plants (**chch**) were mounted on cards with central index tabs. With the exception of **Ch** all the other Mendelian mutants used in this experiment are recessive to the 'wild' type.² On the index tabs was written the symbol for such of these recessive mutants for which the plant was homozygous. An individual of the constitution **chch**, **Oo**, **Tt**, **YY**, **Ff**, **mpmp**, **PP**, **NN**, **CC**, **ZZ**, for instance, would have its photograph mounted on a card with a central tab on which was written '**mp**'. Plants of the constitution **ChCh**, **oo**, **tt**, **Yy**, **FF**, **MpMp**, **pp**, **NN**, **CC**, **ZZ** would require a card with a tab at the left labelled '**otp**'. It was found that this system, once established, was easy to maintain. By means of the notes on the tabs it was possible to index the various mutant combinations and to locate particular records for comparisons. Through the use of this system the junior author in England and the senior author in America have been able to co-operate effectively with a minimum of effort. Over 150 such photographs, representing more than 100 different genetical combinations, have been made and filed, and the collection is still growing. Three record cards are shown in Pl. XIII, Fig. 1. It will be seen that the work has been further simplified by recording the plant number and a black and white 5 mm. scale directly on the photograph.

The chief method of study was to examine families segregating for a particular mutant. If '**o**', for example, were under observation, all the **OO**'s and **Oo**'s on one hand were compared with the **oo**'s as to petiole, blade, bract, peduncle, calyx, and corolla. When tentative conclusions had been reached (an objective which was often hindered by the presence of many other segregating genes) they were checked by referring to the photographic records from other years. Much of this was done in a particular way, which has been illustrated in Plate XIII. The factor in question was compared on different genetic backgrounds. In studying **O** vs. **o**, for example, **ChO** was compared with **Cho**, **pO** with **po**, **tOy** with **toy**, &c. The conclusions reached below, therefore, have been subjected to rigorous tests. They were not arrived at from one set of experiments, but from data gathered in five

¹ The word '*sinensis*' must unfortunately be used in two different ways in this paper. Printed in italics it refers to the species, *Primula sinensis*, to which belong all of the horticultural forms considered in this paper. Printed in bold type it refers to the dominant mutant **sinensis** (**Ch**) which with the recessive **stellata** (**ch**) forms a single allelomorphic pair of genes.

² The real wild type being unknown, we take the **stellata** form of the plant introduced from China in 1820 as our 'wild' type: see Journ. Gen. 27, p. 2 and Pl. XIII.

years of work on plants grown in such diverse environments as Boston, London, and Saint Louis.

PRESENTATION OF DATA.

Oak (oo).

Leaf. The outstanding characteristic of this mutant is the deeply lobed leaf-blade. Making allowances for changes inherent in the lobing, the leaves seem to be otherwise normal. There is no obvious change in the ratio of petiole and blade, the hydathodes are normal in size and distribution, and the shape of the ultimate dentations is unaltered though they are larger and their number is much reduced (Text-fig. 1). *Bract.* The bracts on the



TEXT-FIG. 1. Equivalent portions of the leaf apex from leaves of OO and oo individuals.

average are slightly smaller and less well developed. They occasionally exhibit a slight approach to the trifurcate tip characteristic of the calyx lobe. *Calyx.* The calyx, as a whole, is proportionately smaller and is strongly inverted above, producing a characteristic shape quite different from that of the 'wild' type (Pl. XIII). The individual lobes are more obtuse; this is due to accessory lobes which are more or less developed on either side of the apex of each lobe. In extreme cases this produces a pronouncedly trifurcate tip to each calyx lobe. *Corolla.* The lobes of the corolla are narrower, particularly at the base, producing a star-shaped limb. The notch at the tip of each lobe is deeper, and in flowers with bright coloured petals there is a distinct colourless patch at the base of the notch. On OO plants such a patch is absent altogether or is very inconspicuous. In the presence of certain other mutant genes (*Ch*, *t*, *y*) one or more accessory lobes may be developed at either side of each corolla lobe at its base. These accessory lobes give the flowers of the constitution *ChCh*, *tt*, *yy*, *oo* a very characteristic and bizarre appearance.

Tongue (tt).

Leaf. The outstanding characteristic of this mutant is the narrow, more or less sagittate leaf, which becomes proportionately longer and narrower as it matures. The development of the leaf margin is much affected. The number of ultimate dentations is reduced and they are poorly developed, particularly towards the apex. The hydathodes are developed imperfectly or not at all. The margin of the leaf seems to be undernourished, sometimes it turns brown and dry. In old leaves it ceases to expand, and the continued growth of the main portion of the blade causes the leaf to hump up in the middle and roll under at the edge. The veins are irregular, and microscopical examination shows that conductive tissue is poorly developed

in them, particularly towards the apex. There is a tendency for the production of two small lobes below the main blade of the leaf. When these appear one is usually smaller than the other (or absent altogether), giving the leaf an asymmetrical appearance (Pl. XIV). *Bract.* Bract form is affected by the position of the bract on the peduncle, and since the peduncle may be shortened by *tt* it is difficult to make legitimate comparisons between *TT* and *tt* plants. It is certain, however, that the bracts are less lobed and are proportionately longer in *tt* individuals. *Peduncle.* The peduncle is more or less affected, but this is a point requiring further investigation. '*tt*' plants often have such short peduncles that the flowers do not rise above the level of the leaves. In such plants the number of flowers is also very much reduced, and there may be no more than three per whorl. *Calyx.* The calyx in *chch* plants is very slightly affected. The tip of each calyx lobe is slightly more acuminate than in the *TT* plants. In *ChCh* and *Chch* individuals there is a striking effect. The extra lobing of *Ch* is very much reduced, and the calyx has five recognizable main lobes. Further comment on this point will be found under Corolla and in the general discussion. *Corolla.* In *chch* plants the form of the corolla is only slightly affected, the chief noticeable difference being a slight undulation of the petal margins, and a tendency for adjacent edges to cohere so that the flower does not expand completely. As in the case of the sepal, the lobing of the corolla is very much reduced in *ChCh* flowers. In their effect upon petal and sepal form, *Ch* and *t* seem to be antagonistic, so that *ChChtt* flowers are strikingly similar to those of *chchTT* (Pl. XIII).

Fern (*yy*).

Leaf. In this mutant the leaf blade is decurrent on the petiole. Equivalent portions of the upper parts of the leaf on *YY* and *yy* individuals are approximately similar in lobing and hydathode development. The entire petiole, however, is affected. The decurrent leaf base passes gradually into a small ridge running the entire length of the petiole. Microscopic examination shows that the cells forming the ridges, unlike the epidermal cells of *YY* petioles, are crowded with plastids. *Bract. Calyx. Corolla.* The only effect so far detected in the inflorescence is in the shape of the central eye which in *yy* individuals is straight-sided, producing an eye more like a pentagon and less like a star than is the eye of the 'wild' type. In the presence of other genes which produce lobing of the corolla edge *y* sometimes increases the lobing.

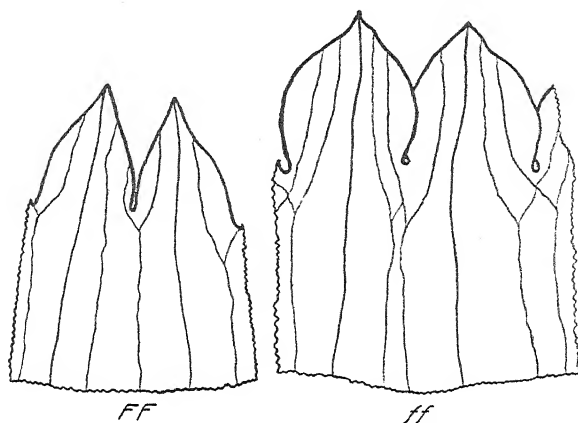
Maple (*mpmp*).

Leaf. The most conspicuous effect of this mutant is upon the shape and texture of the leaf. The lobing is deeper than in the wild type, the number of ultimate dentations is reduced, and the leaves are brittle. The

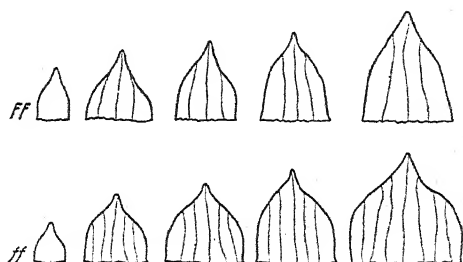
pubescence of the upper leaf surface is defective, the leaves are much thinner, and microscopical examination shows that the palisade cells are iso-diametrical. *Bract*. The bracts have the same abnormal texture as the leaves. *Pedicels*. The pedicels are somewhat shorter than in **MpMp**. *Calyx*. The calyx has the same abnormal texture as the leaves. The calyx lobes are characteristically unsymmetrical. Lobing is much reduced in **ChCh** plants and the calyx has an abnormal contorted shape. *Corolla*. There is no marked effect on a 'wild-type' background.

Crimp (ff).

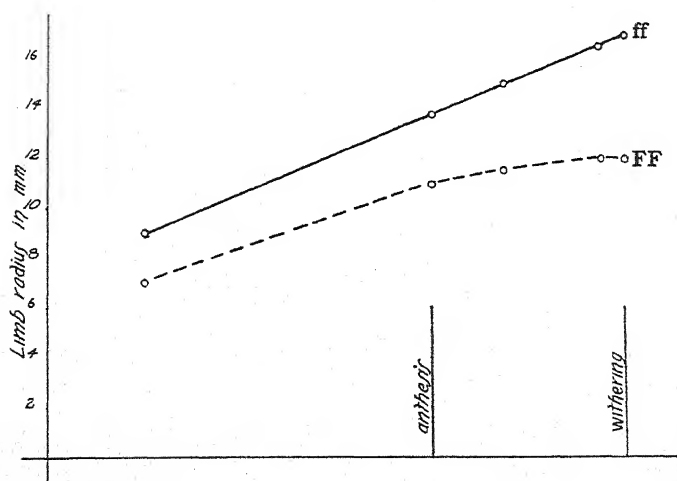
Two recessive allelomorphs to the 'wild' type are known, **f^sf^s**, and **f^lf^l**. The two are very similar, but the former is more extreme in its effects. *Leaf*. In this mutant the most conspicuous effect is upon the leaf margins. The number of ultimate dentations is tremendously increased, although the dentations appear normal and bear normal hydathodes (Gregory, de Winton and Bateson, (5)). The effect is much more marked upon old leaves than upon young ones. In developing leaves one cm. in length there is to the naked eye very little difference between leaves of **ff** and **FF** plants. As the leaves mature the difference becomes increasingly apparent (Pl. XIV). On **ff** plants the leaves continue to develop marginally throughout the life of the plant, and those which persist into the second season of growth on a **ChCh** background become weird globular clusters, superficially quite unlike foliage leaves. It would seem probable, therefore, that the characteristic proliferated margin of crimp plants is due to the fact that in **ff** plants the marginal meristem is active for a longer period than in normal leaves. *Bract*. On the average these are wider and more lobed. *Calyx*. The calyx lobes are much wider at the top. They maintain almost their full width nearly up to the apex where they narrow abruptly into an acuminate tip (Text-fig. 2). *Corolla*. The margin has a strong tendency towards lobing, variously expressed under different genetical and environmental conditions. The lobes are wider and the marginal portion of the corolla is generally more developed. The 'eye' spot of the corolla is very much affected by **f**. The actual effect depends upon the genetic composition of the individual in regard to several other genes. The situation is much too complicated for detailed description here; the whole matter has been discussed by de Winton and Haldane (7). The effect of crimp upon leaf, calyx, and corolla is, on the whole, very similar. It will be noted in Text-fig. 2, and in Pl. XIII that the calyces are similar below; it is in its peculiar development of the margin that crimp calyx differs from the normal. Text-fig. 2 demonstrates that, as in the leaf, the normal and crimp calyces are very similar in their early stages and become increasingly different as growth continues. It is interesting to note that in crimp growth is proportionately more active at the base of the calyx lobes. In



TEXT-FIG. 2. Equivalent portions of the calyx of crimp (*ff*) and non-crimp (*FF*) to show differences in shape and venation.



TEXT-FIG. 3. Comparison of calyx lobe development in crimp (*ff*) and non-crimp (*FF*).



TEXT-FIG. 4. Growth curves of corolla limb in crimp (*ff*) solid line and non-crimp (*FF*), broken line.

the flower it is a simple matter to compare crimp (*ff*) and non-crimp (*FF*) quantitatively. Typical flowers of each were chosen at five stages; (1) in the bud when the corolla first showed colour; (2) when the anthers opened; (3) when the pollen was shed; (4) when withering began; (5) when withering was nearly complete. The radius of the limb was measured and the five measurements from *ff* flowers spaced so that they formed a straight line. Using this arbitrary arrangement, the lengths of the radii in *FF* flowers were then plotted on the same diagram, Text-fig. 4. It will be seen that the rate of growth throughout is higher in *ff* corollas and that it continues higher until the corolla dies. It can be calculated from the curves that the crimp corolla expands 10 mm. after the pollen sacs open, whereas the normal corolla only grows 2 mm. wider during the same period.

Cup (*cc*).

In this mutant the whole plant is much dwarfed, the leaves are small and are so stiffly erect that they form a characteristic funnel or cup. Cup has been so recently added to the experiment that detailed description of its effect upon the inflorescence is not yet available.

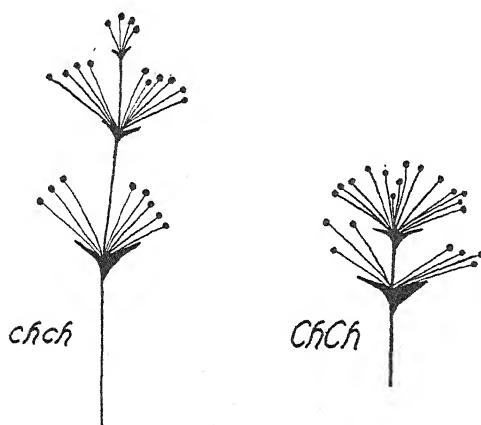
Claw (*zz*).

In this mutant the fundamental ground plan of the leaf is very much upset. It apparently results from the initiation of leaf-tip formation at several points in the embryonic foliar primordium instead of at one. *Bract*. The bracts are long and thread-like. *Corolla*. The corolla has a characteristic fluted appearance like 'egg-shell' note-paper. *Calyx*. It is much affected at the base, producing a trumpet-shaped rather than broadly urn-shaped calyx.

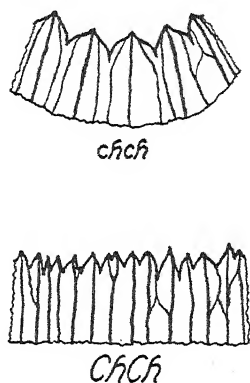
Sinensis (*ChCh*).

The remarkable dominant mutant *Ch*, or 'sinensis', to use the name given it by *Primula* fanciers, has a remarkable effect upon the whole inflorescence. *Leaf*. Except in the case of *ff* plants no effect upon the leaves has been detected. *Peduncle*. The inflorescence appears as if telescoped from above. The lower internode is characteristically much shortened, and even in old well-nourished plants the number of nodes in the inflorescence is seldom more than three, contrasted to the five or more of *chch*. Diagrams, to scale, of *ChCh* and *chch* inflorescences are shown in Text-fig. 5. *Bract*. The bract is larger, broader, and much more lobed. *Corolla*. The eye of the corolla is much larger, the lobes are overlapping and are variously lobed, depending upon other genetic modifiers. *Calyx*. The calyx has the appearance of being much larger. Text-fig. 6, showing the venation in the upper parts of *chch* and *ChCh* calyces, reveals the manner in which this is brought about. The *chch* calyx has three veins for each

lobe, a mid-vein and two lateral veins for each, making fifteen veins, or thereabouts, for the whole calyx. In **ChCh** plants the number of veins remains the same, but there is no differentiation into mid-veins and lateral veins; each vein terminates in a narrow lobe and there are consequently



TEXT-FIG. 5. Diagrams, to scale, of inflorescences of **chch** and **ChCh** plants.



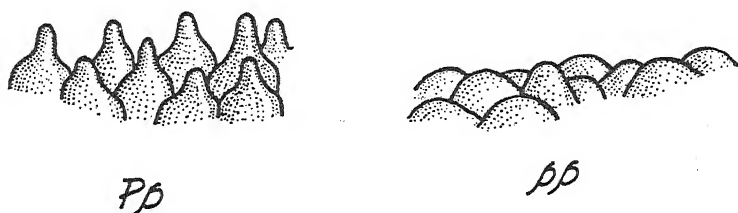
TEXT-FIG. 6. Equivalent portions of the calyx from **chch** and **ChCh** individuals.

about fifteen lobes instead of five. The extra lobing releases the margin and the calyx is no longer contracted at the apex. (This effect is demonstrated in Text-fig. 6, where the dissection of the **chch** calyx is somewhat circular.)

Leafy calyx (nn).

Leaf. Leaves of **nn** and **NN** plants are apparently indistinguishable. **Inflorescence.** **nn** individuals remain for a long time in the rosette stage. When the inflorescence finally appears its bracts and calyces are remarkably leaf-like, particularly below. The bracts at the lowest node may have petioles and blade and resemble normal foliage leaves very strongly. Successive nodes become less and less leaf-like and the bracts and sepals on the terminal nodes of **ch** plants are usually normal. The corollas are usually larger than on **NN** plants but are otherwise normal. To one with some horticultural experience these bizarre mutants are strikingly similar to plants which have been artificially disbudded (Pl. XIII). From the growing points of inflorescences there is apparently given off a substance or substances which, passing backward, inhibits foliar development. Goebel (4) has reported the leaf-like development caused in *Calliandra tetragona* by premature removal of the flower. Similar effects can be produced artificially in *P. sinensis*. If a normal plant is repeatedly disbudded the bracts may become quite leaf-like (Pl. XIV). Leafy calyx, therefore, seems

to be a mutant in which there is what we may loosely call 'internal dis-budding'. The passing from vegetative phase to the reproductive phase, which occurs so rapidly in normal plants, is very much retarded, causing a morphological effect similar to the artificial retardation of disbudding.



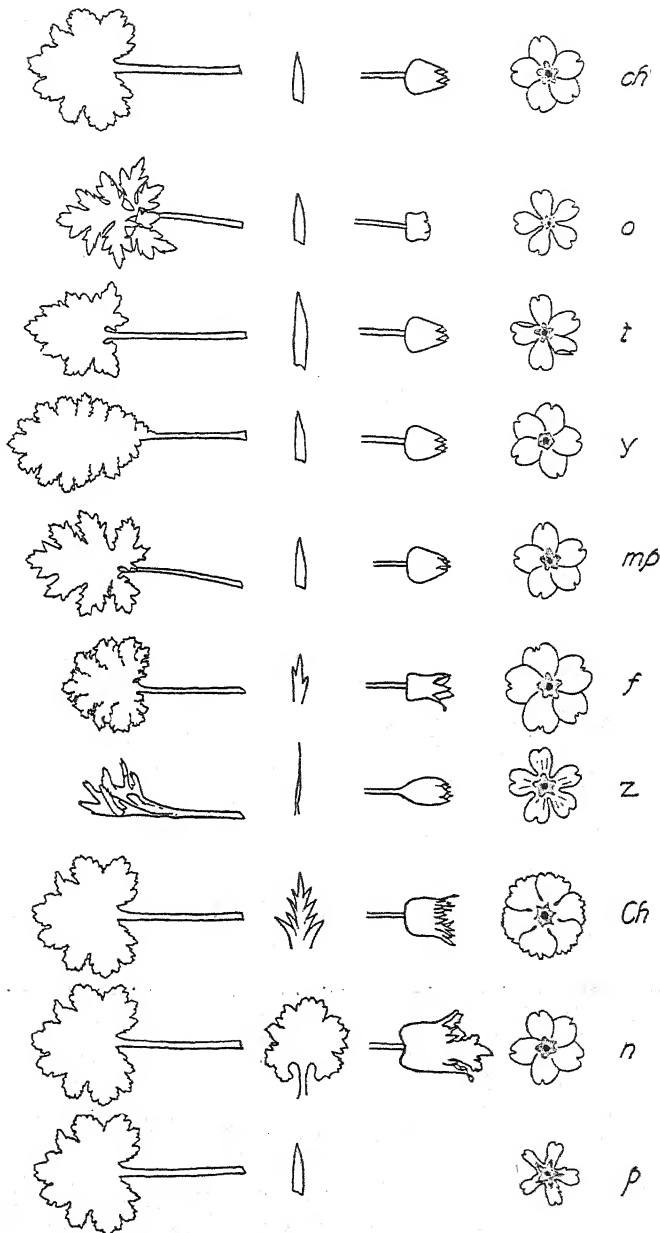
TEXT-FIG. 7. Epidermal cells of *Pp* and *pp* individuals, viewed from the side.

Peculiar eye (*pp*).

Leaf. No effect upon the leaf has been detected. *Bract*. No effect upon the bract has been detected. *Calyx*. The calyx lobes are slightly more acute. *Corolla*. The eye spot is increased, and particularly at the corners it is less definitely delimited, often spreading well out into the blade of the corolla lobe. The corolla is more lobed, the amount and degree of lobing depending upon other factors having a similar effect (*A' f*, *Ch*, *y*). Microscopical examination shows that the epidermal cells of the corolla are much less papillate in *pp* plants. Text-fig. 7 shows cells from comparable regions on *Pp* and *pp* individuals.

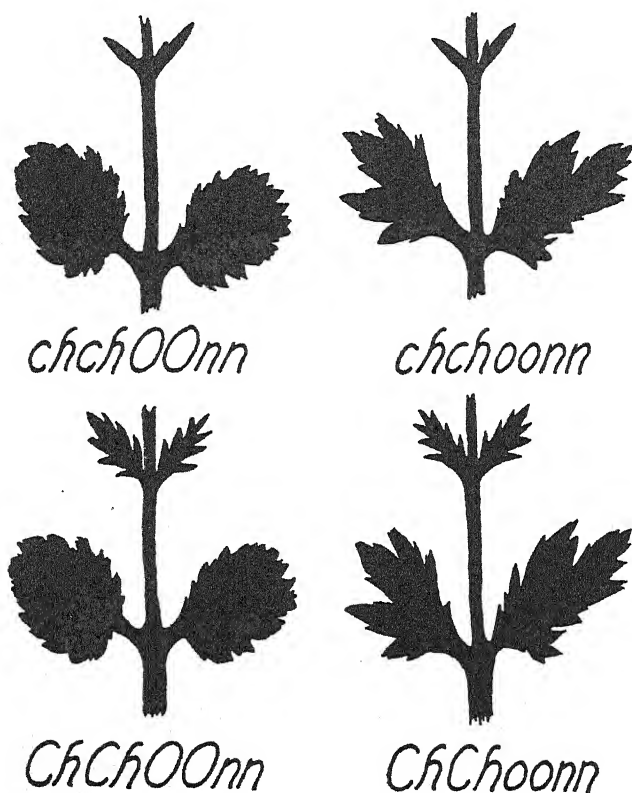
DISCUSSION.

Summarizing the information obtained from a study of all the factors (Text-fig. 8) it is possible to make two generalizations: (1) the factors fall into two classes, those having their chief effect upon the leaf and those having their chief effect upon the inflorescence; (2) Factors affecting leaf shape are found to have supplementary effects upon the inflorescence, but inflorescence factors have no supplementary effects upon the leaves. These two generalizations suggest the following simple hypothesis. *P. sinensis* has a marked division into a vegetative phase and a reproductive phase. Genes operating early enough in development to affect the leaves will quite naturally affect the inflorescence. Genes, however, which mainly affect processes occurring after the plant has passed into the reproductive phase will not affect leaf shape. There is a single important exception to this generalization which, when examined carefully, is found to constitute another argument for the hypothesis. It involves the interaction of *crimp* (*ff*) and *sinensis* (*ChCh*). In spite of its conspicuous effect upon the



TEXT-FIG. 8. Leaf, bract, calyx, and corolla of the 'wild' type of *P. sinensis*, and of nine mutants affecting leaf and inflorescence shapes. All are shown on a wild type background except the calyx of '*nn*' which is shown on a *ChCh* background.

inflorescence, *Ch* has ordinarily no effect upon leaf shape or size. This is true, not only of conspicuous differences such as lobing, size of blade, &c., but of minor tendencies which might be revealed by the repeated examination of segregating families. In spite of painstaking search no such



TEXT-FIG. 9. Diagram showing the effect of the mutant genes 'oak' (*ch*) and 'sinensis' (*Ch*) on the lower and upper whorls of bracts of 'leafy calyx' (*nn*) plants.

differences have been discovered. The one exceptional case is that on a **crimp** (*ff*) background *Ch* plants have leaves which are conspicuously more proliferated along the margin than are *chch* individuals. It will be remembered, however, that **crimp** leaves owe their peculiarity to the fact that they continue to develop along the margin after growth, for normal leaves, has practically ceased. **Crimp** leaves, in other words, differ from the normal in that they continue to develop after the portion of the plant on which they occur has passed from the vegetative phase into the reproductive phase. They are therefore subject to modification by genes such as *Ch* which operate during that phase.

The interaction of the leaf and inflorescence genes in plants homozygous for **leafy calyx** (**nn**) is another proof of the validity of the hypothesis. The situation has been described in detail by de Winton and Haldane (7). It will be remembered that in plants homozygous for **leafy calyx** the lower bracts of the inflorescence are leaf-like, while those of the terminal nodes are normal. It has been found that the leafy bracts of the lower nodes are affected by leaf mutants in much the same way as are the leaves, but are not affected by the flower shape mutants. The bracts of the upper nodes, however, are (like normal bracts) affected only slightly by the leaf mutants yet are strongly affected by the inflorescence mutant **Ch**. As de Winton and Haldane have pointed out (loc. cit., p. 26), 'We have thus the extremely interesting situation that a given organ is controlled by one or another set of factors according to its place on the plant'. The situation is shown diagrammatically in Text-fig 9. The figure illustrates as well a point made in the introduction; that Mendelian mutants provide useful experimental tools for morphological analysis. In this example we are using three such tools. One of them, **leafy calyx** (**nn**), provides a race in which the change from vegetative phase to reproductive phase is through intermediate stages which give some clue as to the nature of the change. On this background, using the other two genes, we may, on the one hand, vary an internal factor operating in the vegetative phase (**OO** vs. **oo**), and on the other one operating in the reproductive phase (**ChCh** vs. **chch**). We find that the leafy bracts at the base react like leaves and not like bracts, and the normal bracts on the same inflorescences react like bracts and not like leaves.

From the behaviour of disbudded plants and of the leafy calyx mutants we can draw a number of useful inferences as to the nature of the change from vegetative phase to reproductive phase in *P. sinensis*. It is apparently due to some substance or substances which come back from the growing point, and which simultaneously inhibit foliar development while stimulating peduncle elongation. Since this substance is affected so profoundly by a single gene it is perhaps a single substance.

Conclusions as to the relationship of leaf, bract, calyx, and corolla.

To one who has come to realize the almost complete absence of information as to the ontogenetic relationship of leaf, bract, sepal and, petal, much of the voluminous literature concerned with the probable phylogenetic relationships of these organs seems premature speculation. When we know something of the developmental relations between these obviously related organs we may then, perhaps, be in a position where such questions can be considered. This point of view has been forcefully developed by Foster (3) in his consideration of the relationship between cataphylls and foliage leaves. His conclusions might well be extended to the more general

problems considered here. 'When the nature of the primitive leaf in angiosperms and the details of its evolution have been inductively established, a phylogenetic interpretation of the cataphyll may be feasible. Until that time we must rest content with the concrete evidence furnished by ontogeny and experimentation.'

Manifold effects of genes.

The study of manifold effects of particular genes is of primary importance in unravelling the complicated story of development in either plants or animals. And although, on the plant side, we may not have as yet the codified information which will enable us to understand the connexion between relationships revealed by this method, its successful use in animal material (Dobzhansky (1, 2); Schultz (6)) recommends it as an explorative tool. Its application to such problems has been discussed by Schultz (6). 'The very fact that a given gene affects two apparently unrelated developmental processes may mean either that an unsuspected relation exists between the two processes or that the gene is capable of exerting more than one primary effect.' From a study of the genes affecting eye colour in *Drosophila* he concludes, 'The interactions as observed appear to be summation products of independent reactions. *Each gene does its own job*. . . In the co-operation of genes all loci are concerned but each effect is specific'.

In *Primula* the working hypothesis that manifold-gene-effects result from some developmental relationship receives confirmation when all such effects are tabulated. If only a single gene were to affect both the eye spot of the corolla and the lobing of the corolla edge, we might argue that the gene in question exerted two primary effects. But when we find upon examination that not only are there several genes with precisely the same combination of effects, but that every gene which effects the eye also effects the lobing, it becomes certain that an important developmental relationship exists between these two regions. The evidence has been discussed in detail by de Winton and Haldane (see in particular their Table VII, p. 17); the salient points can be summarized as follows:

Mutant gene	Effect on 'eye' spot	Effect on lobing of corolla
Ch	increased	increased
y	pentagonal	occasionally increased
p	increased at the base of the lobes	increased slightly at the base of the lobes
a	greatly increased	greatly increased

Another developmental relationship revealed by a study of manifold gene effects is between the elongation of the peduncle and the lobing of the calyx and corolla. There are three genes which affect the peduncle pro-

foundly; all three of them affect the lobing of the calyx, on certain genetic backgrounds at least. The evidence may be summarized as follows:

Mutant gene	Effect on calyx	Effect on peduncle
Ch	increases lobes to one for each vein	peduncles shortened, particularly the upper internodes
t	inhibits lobing of lateral veins	peduncles shortened, particularly the lower internodes
n	lobing increased on lowest internodes	peduncle shortened, particularly on lowest internode

It is interesting to note that whereas in the flower **Ch** and **t** seem to have opposite effects, the one increasing sepal and petal lobing, the other reducing it, they both exhibit an inhibiting effect upon peduncle elongation, though at opposite ends of the peduncle. This would point to a difference in distribution of some regulatory substance. It may be that all three genes affect the production and distribution of a single substance (possibly a growth hormone) which inhibits foliar expansion and promotes peduncle growth.

Passing to a consideration of the manifold effects of single genes, we find most of the cases quite intelligible on the hypothesis that each gene, as Schultz has said, has its own job to do. **Crimp**, for instance, causes marginal growth to persist in the leaves until the death of the leaf. It effects sepal margin and corolla limbs in practically the same way. **Oak** increases the sinuses on both corolla and leaf. **Maple** effects the cuticle in leaf, bract, and sepal. In **tongue** the lateral development of the leaves is inhibited, and a similar effect can be seen in the calyx and corolla lobes. **Claw**, on the other hand, gives no hint as to why there are associated a disorganized leaf-blade, thread-like bracts, a narrow calyx, and a fluted corolla. Nor does the effect of **oak** upon the sepal margin suggest any obvious correlation to its effect upon the leaf. Like the association between peduncle elongation and calyx lobing these dissimilar effects suggest developmental relationships which were previously unexpected, and which are still inexplicable.

We may use these resemblances and differences of reaction to mutant genes to determine the closeness of relationship (ontogenetically) between leaf, bract, sepal, and petal. Fig. 10 shows, for instance, the number of cases in which any two of these organs have a similar response to the same gene change, and lists the genes for each particular comparison. We can even go farther and form correlation tables by scoring like and unlike effects and calculating coefficients of correlation. The data from the ten mutant genes give the following results:

leaf-bract	$\gamma = +0.14$	bract-calyx	$\gamma = +0.67$
leaf-calyx	$\gamma = -0.23$	bract-corolla	$\gamma = +0.33$
leaf-corolla	$\gamma = -0.33$	calyx-corolla	$\gamma = +0.33$

It will be noticed that the closest correlation is between bract and calyx and that calyx and corolla have an unlike reaction from the leaf oftener than they have a like reaction. Closeness of relationship among these four organs could be illustrated diagrammatically about as follows:

	corolla	calyx	bract
leaf	2 <i>f, o</i>	2 <i>f, mp</i>	3 <i>f, t, mp</i>
bract	2 <i>f, Ch</i>	5 <i>f, mp, r, z, Ch</i>	
calyx	3 <i>f, t, Ch</i>		

TEXT-FIG. 10. Number of similar reactions to mutant genes between leaf and corolla, leaf and calyx, etc.

leaf bract calyx corolla.

SUMMARY.

1. The use of Mendelian mutants as variables for morphological and physiological experimentation is discussed. This method is then applied to the study of relationships between leaf and inflorescence in *Primula*.
2. Detailed descriptions are given of the effect of ten mutant genes upon leaf, bract, calyx, and corolla.
3. These genes fall into two classes, those having their major effect upon the leaves and those having their major effect upon the inflorescence. Genes in the former group have supplementary effects upon the inflorescence but genes in the latter group do not effect the leaves.
4. The following hypothesis is put forward to explain the facts summarized in 3: *P. sinensis* has a marked vegetative phase followed by a reproductive phase. Genes operating in the vegetative phase will have a supplementary effect upon the reproductive phase which follows. Genes which do not come into play until the plant is in the reproductive phase will have no visible effect upon the vegetative organs.
5. The change from vegetative phase to reproductive phase is brought about by a regulatory substance which simultaneously inhibits foliar development and stimulates peduncle elongation.
6. Through the analysis of manifold gene effects an ontogenetic relationship is demonstrated between (a) corolla lobing and the development of the 'eye' spot, (b) calyx lobing and peduncle elongation.
7. Using likeness and unlikeness of reaction to mutant genes, correlation coefficients are calculated between leaf, bract, calyx, and corolla. The closeness of relationships between these four organs ontogenetically can be roughly diagrammed as follows:

leaf bract calyx corolla.

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EXPLANATION OF PLATES XIII and XIV

Illustrating the paper by Dr. E. Anderson and Miss D. de Winton on 'The Genetics of *Primula sinensis*. IV'.

PLATE XIII.

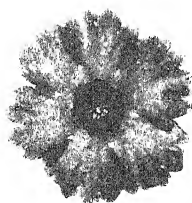
Fig. 1. Three record cards showing dissections of calyx, sepal, and bract. Above: 'wild' type (chchTT). Centre: *sinensis* (ChChTT). Below: *tongue sinensis* (ChChtt).

Fig. 2. Above: effect of fern(yy) on a *sinensis* background (ChCh) and an Oak background (oo). Below: calyces of 'wild' type (chch), oak(oo), and *crimp*(ff).

PLATE XIV.

Fig. 3. Above: young and mature leaves of *crimp*(ff) and 'wild' type (FF). Below: asymmetrical leaves of *tongue* (tt).

Fig. 4. Above: leafy bracts produced by repeated disbudding of a normal plant. Below: modification of leafy bracts by genes affecting leaf shape. Left to right: leaves (below) and leafy bracts (above) of fern, 'wild' type, and oak. The two small bracts to the left and right of the centre are normal bracts for comparison.



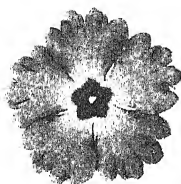
yCh



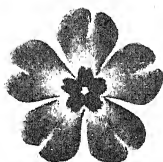
yo



ff



YCh

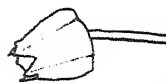


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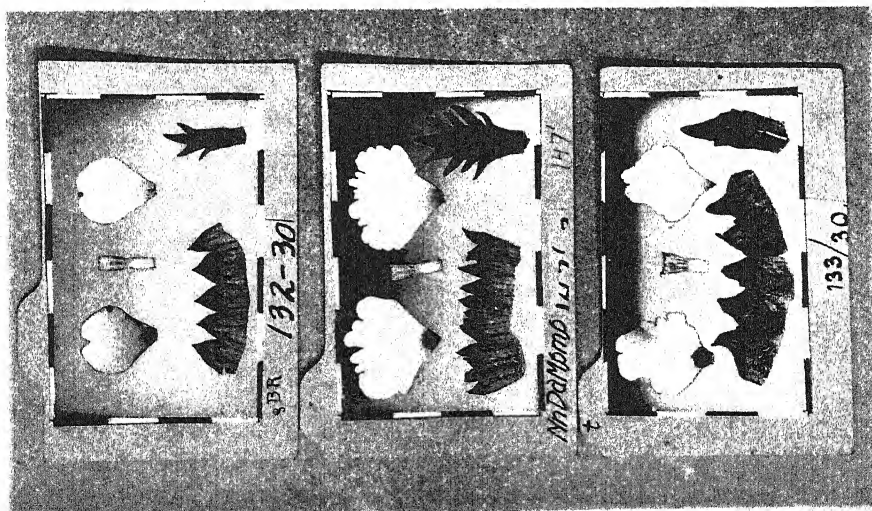


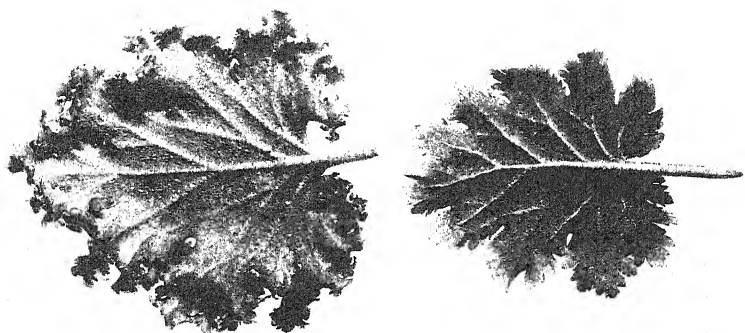
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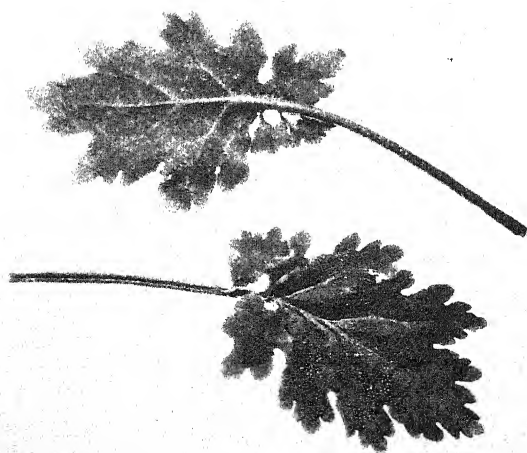


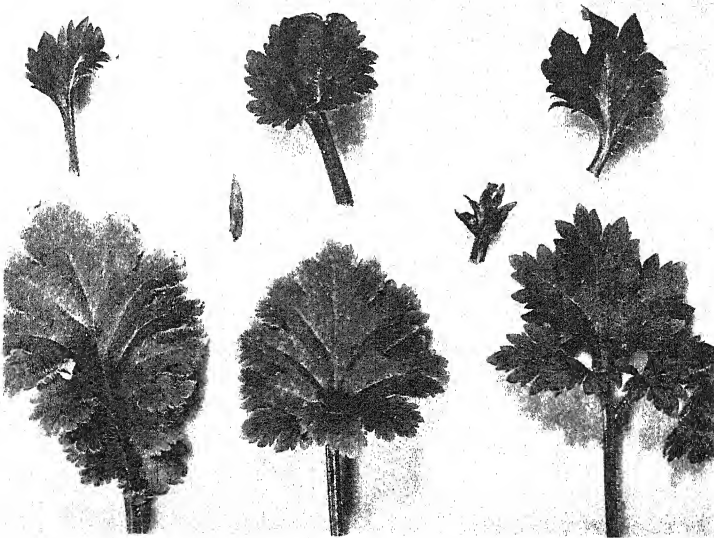
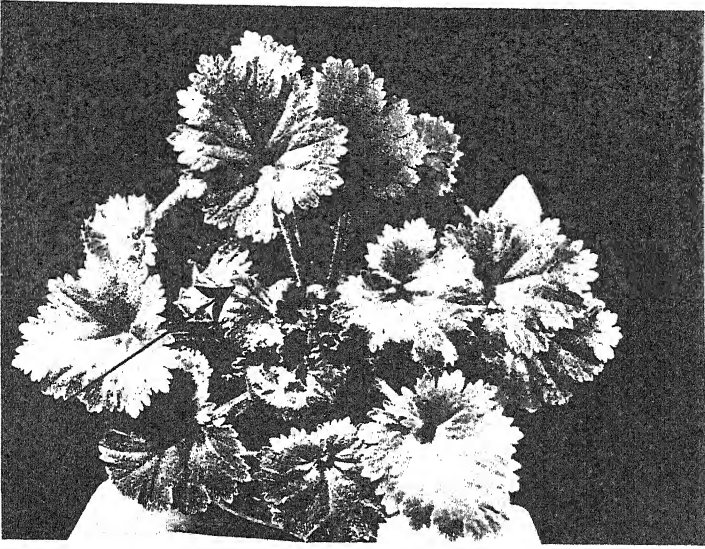


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Half-disjunction in an Association of Four Chromosomes in *Pisum sativum*.

BY

E. SUTTON.

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With seven Figures in the Text.

I. INTRODUCTION.

THE occurrence of an interchange of segments between non-homologous chromosomes in plants was first suggested by Belling (2) as an explanation of partial sterility previously recorded by him in *Stizolobium* (1). The hypothesis has since proved adequate in interpreting further observations on other plants.

If two non-homologous chromosomes made up of segments AB and CD respectively exchange segments B and D, the resulting chromosomes will be AD and BC respectively. In a plant in which there are two normal chromosomes whose homologues have exchanged segments in this way (an interchange heterozygote) the interchanged chromosome AD will be able at meiosis to form chiasmata in segment A with segment A of AB, and in segment D with segment D of CD. Similarly the two segments of BC will be able to form chiasmata with the corresponding segments in AB and CD. An association of four chromosomes can therefore be formed, and such associations replacing two bivalents have been found in *Datura* hybrids (3, 4), in *Zea* (5, 6, 16) in *Campanula* (11), and in *Pisum* (12-15, 17). In addition, associations of three and five chromosomes have been found in 'tertiary' trisomics of *Datura*, where the extra chromosome has an interchanged segment (4); and in *Oenothera* up to the full complement of fourteen chromosomes may be involved in a multiple association.

In *Pisum*, seven cases of segmental interchange have been described (12-15, 17), the interchange heterozygotes (in the hybrids with a normal line) having an association of four chromosomes plus five bivalents at the first meiotic division, instead of the usual seven bivalents. These interchange lines have not all been tested against one another; but by crossing two of them together (T line \times K line) an association of six chromosomes has been obtained (18), a result not to be expected from crossing plants

homozygous for the same interchange (see below), and therefore showing that these two interchanges are distinct.

The following relates to an association of four chromosomes, accompanied by approximate half-sterility, found by Professor Winge in hybrids between a normal line (N line) and a line with violet-coloured flowers, and subsequently grown by Miss Pellew. Interchange homozygotes of the Winge line have been crossed with those of the T and K lines, giving in both cases approximately 75 per cent. sterile plants with associations of six chromosomes at meiosis (Sansome, unpublished) and showing that the interchange is not identical with that in either of these lines.

2. METHODS.

I am indebted to my colleague, Mr. Fabergé, for handing over to me slides showing pollen mother-cell divisions from a plant 88⁷/33. This was a half-sterile plant from 49³/32, which was grown from seed of a half-sterile F₂ plant from Professor Winge's cross between violet-flowered and emerald-green lines. Flower-buds were fixed in Carnoy's fixative and La Cour's 2B, and the sections were stained by the gentian violet-iodine method.

3. MEAN CHIASMA FREQUENCY AND TERMINALIZATION COEFFICIENT.

The mean chiasma frequency per bivalent (counting only the five bivalents, not the associations), the mean frequency per chromosome (counting all seven), the mean frequency per chromosome in the associations, and the terminalization coefficient (number of terminal chiasmata/total number of chiasmata) found from observations on ten pollen mother-cells at metaphase of the first meiotic division, are shown in the table.

											Total.	Mean ch. freq.	Term. coeff.
Total chiasmata per cell . . .	17	18	18	19	19	19	20	21	21	21	193	2.76	—
Chiasmata per 5 bivalents . . .	13	14	15	13	14	13	15	15	15	17	144	2.88	—
Chiasmata per association of 4	4	4	3	6	5	6	5	6	6	4	49	2.45	—
Terminal chias- mata per cell .	7	8	7	9	8	11	9	11	12	10	92	—	0.47

The values of 2.88 and 0.47 are higher than those found by Mrs. Sansome (17) for the T line interchange heterozygote (2.55 and 0.36). The parent plants in the one case were N line and Winge line, in the other N line and T line. It is not known whether chiasma frequency and terminalization coefficient differ in the T and Winge lines or in the N line parents; this may account for the different values in the F₁'s from crosses with N line plants. Otherwise it may be that multiple association affects chiasma formation in the remaining bivalents.

4. THE ASSOCIATION OF FOUR CHROMOSOMES.

Two of the four associated chromosomes have submedian, the other two subterminal, attachment constrictions or centromeres (cf. Darlington (9))



FIG. 1.

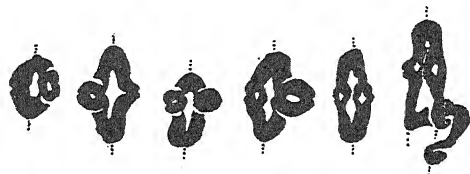


FIG. 2.

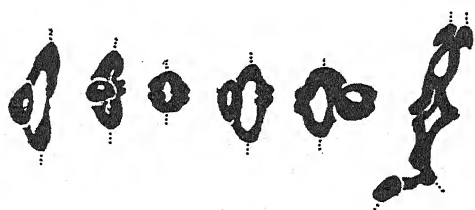


FIG. 3.

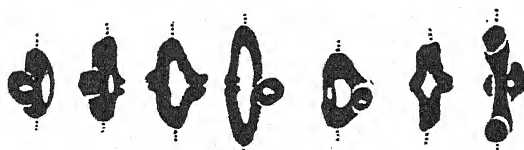


FIG. 4.

FIGS. 1-4. Side-views of first metaphase in pollen mother-cells. 1. With association of four chromosomes giving half-disjunction at anaphase. 2. With chain of four giving numerical non-disjunction. 3. With association of four giving non-disjunction, homologous centromeres directed towards the same pole. 4. With seven bivalents. \times ca. 3000. (The associations of four are represented diagrammatically in Fig. 7.)

(Figs 1-4). Of 161 pollen mother-cells counted at metaphase of the first meiotic division, twenty-two contained seven bivalents (Fig. 4), and in some of these cells bivalents were observed in which the chiasmata on one side of the centromere were unterminalized at metaphase, the ends being unpaired. It seems, therefore, that there is only a small interchanged segment, situated at the end of the shorter arm of the chromosome with submedian centromere, and of the long arm of that with the subterminal centromere; since

the short arms of the latter are able to form chiasmata with one another (Fig. 6 *b*) and are therefore homologous. The major part of each chromosome still pairs as in the original non-interchanged bivalent.

The number of chiasmata in the association varies from three to six. The mean chiasma frequency per chromosome for the two chromosomes involved was found to be 2.45 (see Table), which is considerably lower than that for the rest of the chromosomes.

The commonest configuration is one in which the two chromosomes with subterminal centromeres pair together interstitially in the long arm, and sometimes terminally or subterminally in the short arm (this gives a 'figure-of-eight'), and have terminal or subterminal chiasmata with the other two chromosomes, which also form one or two chiasmata with one another in the other arm. This type of association was observed ninety-nine times (Figs. 1, 6 *b*, *c*).

The 'figure-of-eight' involving four chromosomes only must not be confused with the 'figure-of-eight' of six chromosomes described in maize (7) and in *Pisum* (18). In the present case the configuration results from a chiasma having been formed in the region between the point of interchange and the centromere, and it indicates that this is a fairly big region. If a second interchange took place between this chromosome and a third chromosome, the point of interchange this time being on the other side of the centromere, then the region between the two points of interchange would form the 'interstitial segment' associated with the 'figure-of-eight' of six chromosomes in the double interchange heterozygote. Darlington (8) suggests that the 'differential segments' in *Oenothera* have arisen in this way.

Where a 'median chiasma' is formed between the point of interchange and the centromere, 'half-disjunction' or 'chromatid non-disjunction' will occur owing to an exchange between chromatids and crossing-over between the interchanged segments at this point. From such an association of the chromosomes AB.BC.CD.DA, gametes AB.CD and AB.DC (viable) or AB.BC and AD.DC (deficient and inviable) will be formed in equal numbers; there will always be disjunction of homologous centromeres (Fig. 5).

The next commonest configuration, observed thirty-four times, was a chain of four (Figs. 2, 6 *e*, *f*, *g*), the break usually being between the two chromosomes with subterminal centromeres, owing to the failure of chiasma formation in the short arm. If the separation of the chromosomes at anaphase were completely random, we might expect six kinds of gamete from the chain or unbroken ring with no 'median chiasma', namely, AB.CD and AD.BC (viable) from disjunction of adjacent chromosomes; AD.AB and BC.DC, AB.BC and AD.DC (deficient and inviable) from non-disjunction; besides these types, $(n+1)$ and $(n-1)$ chromosomes might occur in some gametes as the result of numerical non-disjunction (three chromo-

somes going to one pole and only one to the other). All these types have been recognized in maize, where the chromosomes are distinguishable from one another (6, 16). This, however, would give $2/3$ inviable gametes and only $1/3$ viable, whereas it is usual for half of the gametes to be sound

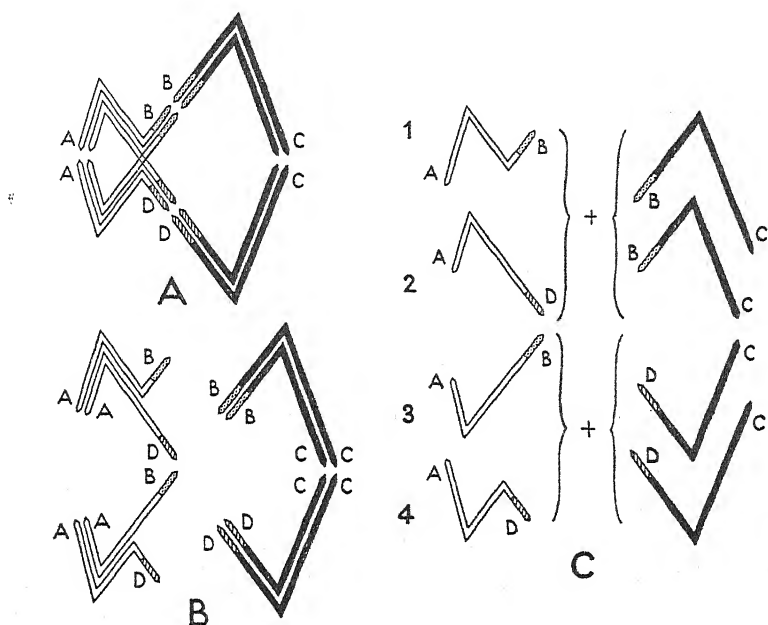


FIG. 5. Diagram illustrating half-disjunction. A. Metaphase configuration showing median chiasma and crossing-over of the interchanged segments of chromatids AB and AD. B. Anaphase configuration showing half-disjunction, the interchanged chromatid segments which have crossed over disjoining from their adjacent homologues, the other two not disjoining. C. Combinations of chromosomes at second meiotic division, 1 and 4 contribute to deficient and non-viable gametes. 2 and 3 to viable gametes.

(13). Fifty per cent. viable gametes would be obtained if non-disjunction were limited in some way—for example, if homologous centromeres always passed to opposite poles, as they might if the repulsion between them were strong (10). Then, supposing the centromeres to lie in segments A and C, disjunction would still give AB.CD and AD.BC gametes, while only non-disjunction of the type giving AB.BC and AD.CD gametes would occur. Where 50 per cent. sterility is found, assortment of the chromosomes seems to be not completely random, but there is randomness as between disjunction and non-disjunction. In *Datura* fully fertile plants having an association of four chromosomes have been found (4), and regular disjunction may be ensured by the median position of the centromeres and the high degree of terminalization of chiasmata, which give the chromosomes more freedom and flexibility. In the *Pisum* chains described, both kinds of non-disjunction occur (Fig. 6 *f, g*) in about equal numbers, and can be distinguished

owing to the different positions of the centromeres. Non-disjunction was found in eighteen cells, regular disjunction in fourteen (Fig. 6*e*), and 'numerical non-disjunction' in two (Fig. 2).

Twenty-two cells with seven bivalents were also observed. Random

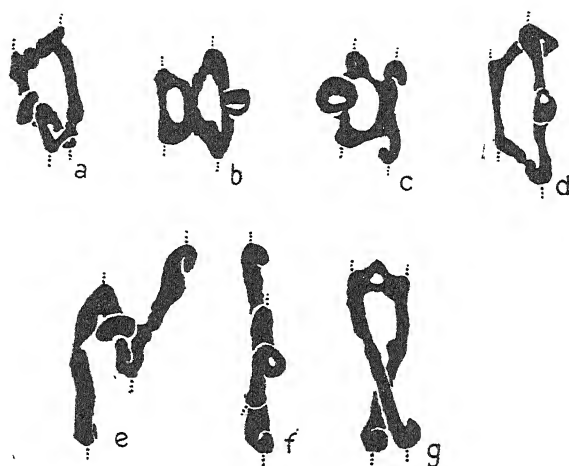


FIG. 6. Associations of four chromosomes from different pollen mother-cells at first metaphase. *a*, open ring, giving non-disjunction, homologous centromeres directed to same pole; *b*, figure-of-eight giving half-disjunction at anaphase; *c*, configuration giving half-disjunction; *d*, open ring giving non-disjunction, homologous centromeres directed to opposite poles; *e*, a chain giving regular disjunction; *f*, chain giving non-disjunction, homologous centromeres directed to opposite poles; *g*, chain giving non-disjunction, homologous centromeres directed to same pole. \times ca. 3000. (These associations are represented diagrammatically in Fig. 7.)

assortment of chromosomes from two such interchange bivalents replacing the association of four will give AB.CD and AD.BC or AD.CD gametes in equal numbers; in this case homologous centromeres must disjoin.

Six open rings all showed two adjacent chromosomes going to the same pole. The two might have homologous centromeres (Fig. 6*a*) or otherwise (Fig. 6*d*).

The chromatid structure of the associations in Figs. 1-4 and Fig. 6 are shown diagrammatically in Fig. 7.

5. GENETICAL CONSEQUENCES.

The numbers of the different types of association found were:

99 closed rings giving half-disjunction at anaphase.

34 chains of 4-14 giving regular disjunction.

18 giving non-disjunction (2 types).

2 giving numerical non-disjunction.

22 cells with 2 bivalents replacing the association of 4.

6 open rings giving non-disjunction (2 types).

These numbers indicate that the plant may have been slightly more than 50 per cent. sterile on the male side. Unfortunately, pollen counts were not made, and the plant set little seed, so that this cannot be checked.

Numerical non-disjunction should give an occasional viable ($n+1$)

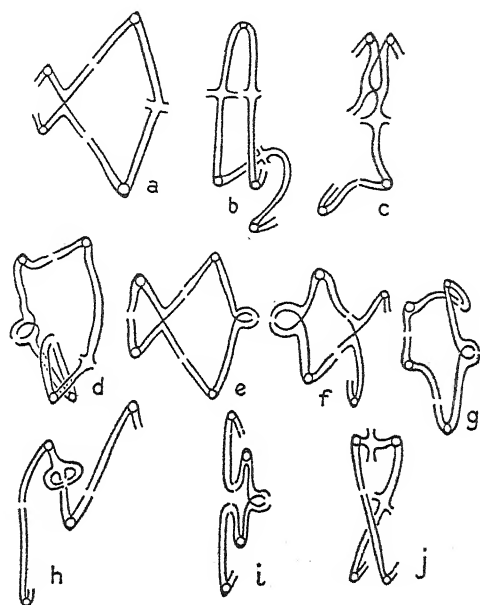


FIG. 7. Diagram of chromatid structure of associations in Figs. 1-3 and Fig. 6. *a-c.* correspond to Figs. 1-3. *d-j* represent Fig. 6 *a-g*.

gamete, and consequently a trisomic among the progeny, but these seem rare, as the examination of several cells at the second meiotic division showed no 8- or 6-chromosome cells.

All viable gametes except ($n+1$) chromosome gametes are either AB.CD or AD.BC. When an interchange heterozygote is selfed, it can therefore give rise to fertile normal (AB.CD) homozygotes, interchange heterozygotes (AB.BC.CD.DA), and fertile interchange (AD.BC) homozygotes. When crossed back to N line, only normal plants and interchange heterozygotes are expected.

If a plant homozygous for an interchange between chromosomes AB and CD be crossed with one homozygous for an interchange between CD and EF (AD.BC.EF \times AB.CF.ED) the F_1 will be expected to show at meiosis an association of six chromosomes (AB.BC.CF.FE.ED.DA) and will be about 75 per cent. sterile. This result has been obtained in *Campanula* (11), *Datura* (4) and *Pisum* (18).

The fact that the Winge line gives approximately 75 per cent. sterility and an association of six when crossed with the K or T line (see above)

shows that the interchange is different, but that one chromosome involved is homologous with one of those in the K line association, and one (perhaps the same one) homologous with one of those in the T line association.

6. SUMMARY.

An association of four chromosomes, in a hybrid between a normal line in *Pisum* and a pure line found by Professor Winge to give half-sterile F_1 on crossing with normal varieties, is described. A small terminal segment has been interchanged between two non-homologous chromosomes.

A chiasma is commonly formed between the centromere and the point of interchange in one pair of chromosomes in the association, and the interchanged segments cross over, resulting in half-disjunction at anaphase and 50 per cent. gametophytic abortion.

The mean chiasma frequencies per bivalent and per chromosome in the plant investigated were 2.88 and 2.76; the mean chiasma frequency per chromosome in the association was 2.45; and the terminalization coefficient was 0.47.

I am much indebted to Mrs. E. Richardson Sansome for her help during the course of this work, to Dr. C. D. Darlington for valuable criticism, and to Mr. H. C. Osterstock, who is responsible for the diagrams.

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The Structure of *Lophodermium pinastri* (Schrad.) Chev.

BY

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With twenty Figures in the Text.

LOPHODERMIMUM PINASTRI is a parasitic fungus which causes the disease commonly known as Pine Blight or Leaf-cast of Pines and is responsible for considerable losses due to premature defoliation. Though it attacks various species of pines, young and old, it is generally agreed that the disease is essentially one of young trees, plantations of one to five-year-old seedlings often suffering heavily and not infrequently being destroyed. The early symptoms of disease (Fig. 1, A) consist of small brown discolorations on the leaves, on which will appear in due succession the minute black pycnidia and apothecia. Whilst young seedlings under severe conditions may sometimes be destroyed and the fruiting bodies developed in the same year of infection, the fructifications usually appear on fully matured needles below the growth of the current year, the pycnidia in the autumn followed by young apothecia, on leaves still on the tree, the apothecia completing their development by early spring or later, on the fallen leaves (Fig. 1, D).

It is the aim of the present paper to trace the development of the fungus in relation to its occurrence on *Pinus sylvestris* and to describe certain observations not previously set forth dealing with the structure and function of the pycnidia as spermogonial organs, especially in relation to the apothecia which succeed them together with details of development in the apothecia themselves.

The disease has received considerable attention from numerous investigators, the work of Carl von Tubeuf (25) chiefly on biological lines, being the most important. This author has also described and figured the gross features of the apothecia and their mode of dehiscence. Prillieux (21) also gives descriptions of the pycnidia and apothecia. Other early accounts are concerned with the incidence of disease and time of appearance of mature apothecia. Prantl (20) observed that the needles fall before the apothecia are developed. Tubeuf (25), Prantl (20), and Haack (12) confirmed parasitism by infection experiments. Hartig (14) declared that the pycnidia

(he also calls them spermogonia) appear in early autumn followed by early stages of apothecia. Recent contributions are those of Hagem (13) and Dufrenoy (8) on the nature and etiology of the disease, and (Wille (26, 27) on the correlation of hydrogen-ion concentration of cell-sap and infection. Langner (18) in his studies of mycelial growth makes the highly interesting statement that this species is probably heterothallic. He observed that the mycelia from monosporic cultures differed in colour from white to brown, and there was also a distinction in respect to their reciprocal attraction or aversion towards each other. From these phenomena he concluded that the species is probably heterothallic. This statement will be discussed in relation to the observations set forth below.

The investigators cited are at some variance as to the time of incidence of disease and also the appearance of pycnidia (spermogonia) and apothecia on the leaves. Several factors are involved which would account for this disparity in the time of appearance of the fructifications. Tubeuf (25) observed that on the older primary leaves and on the double needles if these were injured or in a starved condition, pycnidia and apothecia could be seen in the same year of infection whilst these were still on the tree, adding, however, that normally apothecia occur on the fallen leaves. In agreement with Hartig (14) it is the writer's experience that early stages of apothecial development soon follow upon maturity of the pycnidia whilst the leaves are still attached, and except in rare cases which can be traced to injury of leaf or axis, fully developed apothecia are not seen on the double needles on the tree, these fructifications completing their development on the ground. In this connexion it is interesting to report that Professor J. S. Boyce¹ sent to the writer fruiting specimens of this disease on needles of a tree (*Pinus contorta*, collected at Waldport, Lincoln Co., Oreg.) which had been killed by the Pitch Moth. Whilst it is not believed that such injuries as partially hanging branches or those due to frost or other agency are concerned with infection, the evidence seems to be in favour of the view that they are responsible for setting up changes in the nutriment of the fungus as are found under natural conditions in the fallen leaves and which are favourable to maturation of the apothecia. There is definitely no further infection of the fallen needles, this being also the view of Tubeuf (25) who states that the leaves are not attacked saprophytically on the ground.

Methods.

The writer has had this disease under observation in several plantations and localities in North and Mid-Wales and for several years made a close study of it at Ponterwyd, Cardiganshire. During the whole period of

¹ Professor Boyce also reported *L. pinastri* on *P. ponderosa* and *P. Lambertiana*, both collected at Plumar Co., California, and on *P. radiata*, from Golden Gate Park, California.

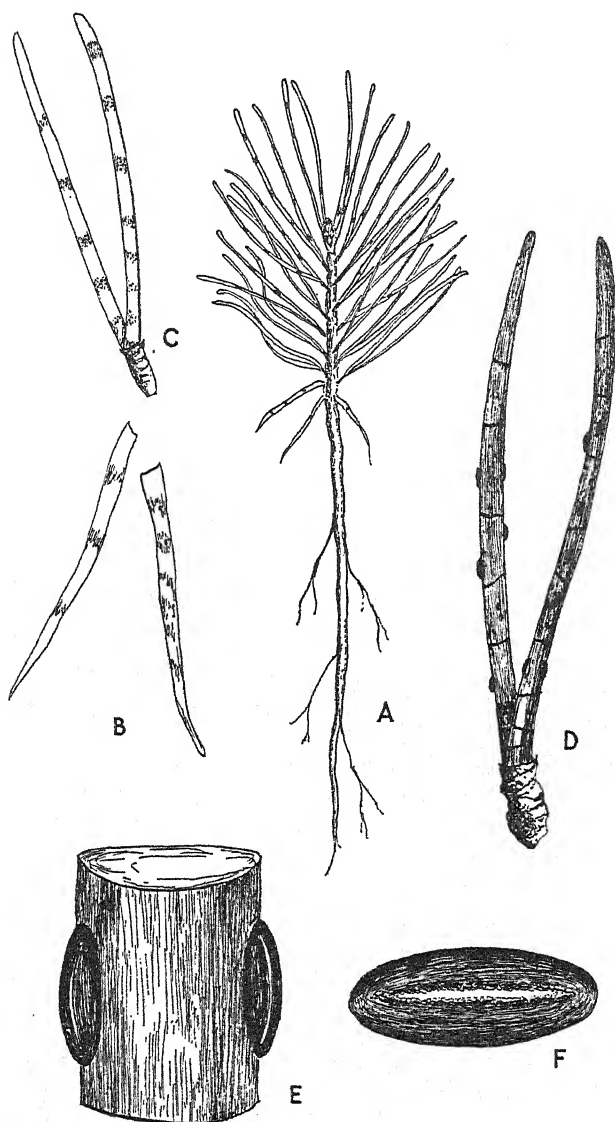


FIG. 1. A. Seedling of *P. sylvestris* at early stage of infection; spermogonia on the primary leaves; spots of early infection on the double needles. B. Two primary leaves, on left young, on right older stage of disease with spermogonia. C. Spots of early infection on a double needle as in A. D. A double needle with mature apothecia. E. Two mature apothecia at the leaf angles. F. A dehiscent apothecium.

observation the trees in this locality never showed the presence of any other diseases which could be confused with the one now described. This point must be emphasized because the whole investigation has been carried out cytologically with material collected repeatedly from the same source. This had to be resorted to owing to the usual difficulties attendant upon the growth of specialized parasites in artificial culture. As Flemming's fluids seem to be unsuitable for the fixation of pine leaves, acetic alcohol was employed; sections were cut to the thickness of 4μ and stained with Heidenhain's iron-alum haematoxylin with or without Congo red (3) as a counter-stain. For the staining of mycelium in the tissues of the host Cartwright's (4) safranin and picro-aniline-blue proved very effective.

Spore Discharge and Germination.

Copious emission of ascospores can usually be obtained in early May if the affected needles are collected from damp situations in the wood or below the surface of a thick deposit of leaves around the trees. If the needles are somewhat dry they can be induced to shoot their spores if placed on moist filter paper in a Petri dish and left for a few hours. As soon as they are withdrawn the apothecia are seen to open along a longitudinal line of dehiscence (Fig. 1, F) in the ridge, this being followed by discharge of a small cloud of ascospores. If the needles are restored to the dish the same apothecia can be used several times for spore discharge. The ascospores, filiform in shape and furnished with gelatinous sheaths, vary in dimensions from 90 to $140\mu \times 1.5$ to 1.7μ . Fixed and stained spore-preparations were made by holding shooting apothecia over a dish of Flemming's weak fluid, a half-hour fixation producing the best results. By virtue of their gelatinous sheaths the spores can be picked up on to the surface of a glass slide brought into contact with the fluid, and the slides can be washed and treated in the usual way. Stained with iron-alum haematoxylin they are seen to be unicellular, the single nucleus occupying a somewhat central position within a constricted part of the cell, this appearance being really due to the inner spore-wall being thickened in hour-glass fashion just around the nucleus (Fig. 18, A, B). When the spores are thus fixed immediately upon their discharge they have not been seen other than in the uninucleate condition. If, however, they are examined in water, even after brief immersion, it is not unusual to see them with two or more nuclei as described by Rehm (22) and Ludwig (19).

The ascospores were cultivated in a weak decoction of prunes and monospore cultures prepared by the usual method of subdilution into agar media. Germination usually proceeds from or near the blunt end of the spore (Fig. 2), the first stage being rupture of the gelatinous sheath, followed by a short germ tube, the latter soon expanding into a large vesicle. These

early stages of germination lifted from a liquid medium were fixed and stained in the same way as the spores. The vesicles are seen to be multinucleate, and there is now no sign of any nuclei in the spore itself, though its contents are still very granular. Growth is continued from the vesicle

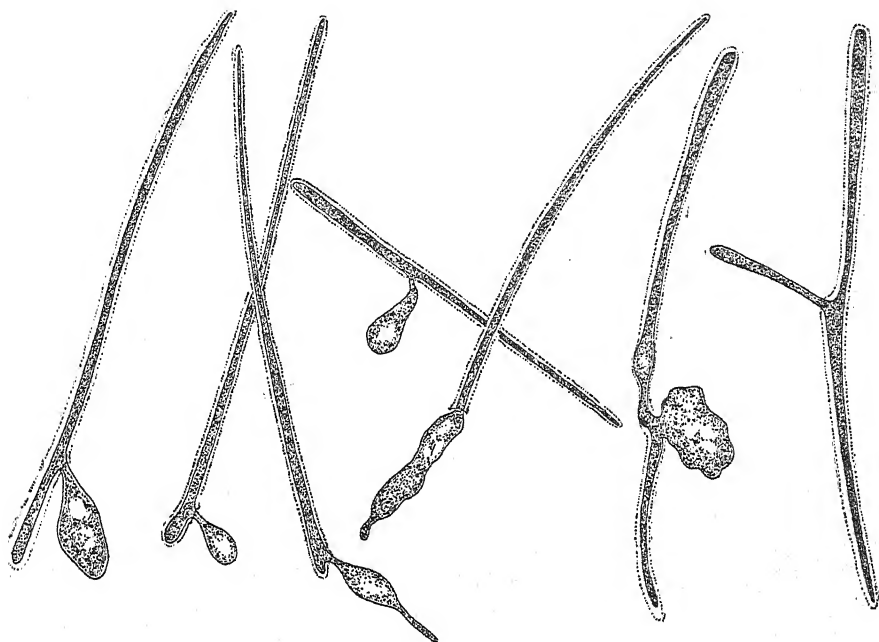


FIG. 2. Stages of ascospore germination; penetration of spore-sheath; note multinuclear contents of vesicles.

by the production of one or more germ tubes; some spores grow without the intervention of a vesicle, but they are relatively few. On the artificial media growth soon comes to an end and the writer has not succeeded in getting the fungus to fruit in culture.

Early Infection.

The first visible signs of infection on the leaves are seen usually towards the end of June, as small greyish areas situated at varying intervals along the leaves. The colour, however, varies considerably, being sometimes yellow or tinged with purple, but a brownish colour is always good evidence of well-established infection. In young plantations the first leaves to be affected are the primary (Fig. 1, B) followed by the double-needles from below upwards. This observation seems to be in agreement with the fact that infection takes place from spores conveyed by air currents from the old leaves on the ground. All observers agree that the disease attacks

mature leaves only, and there are no signs of infection in the tissues of the young needles on the opening shoots.

The stained preparations of the leaf-sections reveal beyond doubt that penetration takes place by way of the stomata. The mycelium creeping over the leaf-surface (Fig. 3) is somewhat coarse, thick-walled, septated, and of a yellowish colour. It is a frequent occurrence to see one or more hyphae entering the same stoma, whether these are derived from multiple infection or consist of hyphal branches could not be determined, but the material being of small compass is exceedingly favourable for the study of stomatal penetration. These early stages are abundant even in a single preparation, and in an infected spot there are relatively very few stomata that are not exploited. Within a stomatal pit the invading hypha or hyphae are always accompanied by a deposit of homogeneous substance of the nature of yellow gum which stains black in the preparations; it seems to consist of the dilated gelatinous spore-sheath, but it is difficult to make out its exact structure owing to the amount of debris adherent to it.

The invading hyphae are now seen to approach the guard cells, sending out into one or both a fine delicate branch which soon forms a small plexus within. Continuing its growth the invading hypha now in the substomatal space becomes considerably dilated, appearing almost vesicular, thus repeating the features exhibited by the spore-germlings. The vesicle immediately puts forth a number of branches which frequently coil around and obscure it, the whole body eventually becoming covered over with deposits of black substance. A similar feature is described by Killian and Likhite (17) in the stomatal infection of *Crataegus monogyna* by *Lophodermium hysterioides* and called by them a 'sclerote'.

The fungus having thus established itself in the sub-stomatal cavities proceeds again to form rather coarse yellow hyphae, but these soon branch into a finer and colourless kind in which septation into uninuclear vacuolated cells is evident. The delicate mycelium soon enters the mesophyll causing this tissue to become disintegrated very rapidly, and though the cell contents are absorbed the invasion is initially intercellular and is accomplished without the aid of haustoria.

Despite the disintegration of the mesophyll, digestion of its cell-walls is not progressive, and in parts the membranes appear swollen and heavily impregnated with black substance. A striking feature is the way in which the endodermis appears to check invasion into the stele which, with its tissues clear and intact stands out in sharp contrast against the almost structureless mesophyll for a considerable time (Fig. 4).

However penetration of the endodermis is eventually accomplished, and after exploring the mesophyll the tips of the invading hyphae are seen to collect largely opposite the radial walls of the endodermis; comparatively few hyphae are seen impinging on the tangential walls. In both cases the

hyphal tips are coated with black substance giving the appearance of small caps or wedges directed against the cell-walls. The blackening also

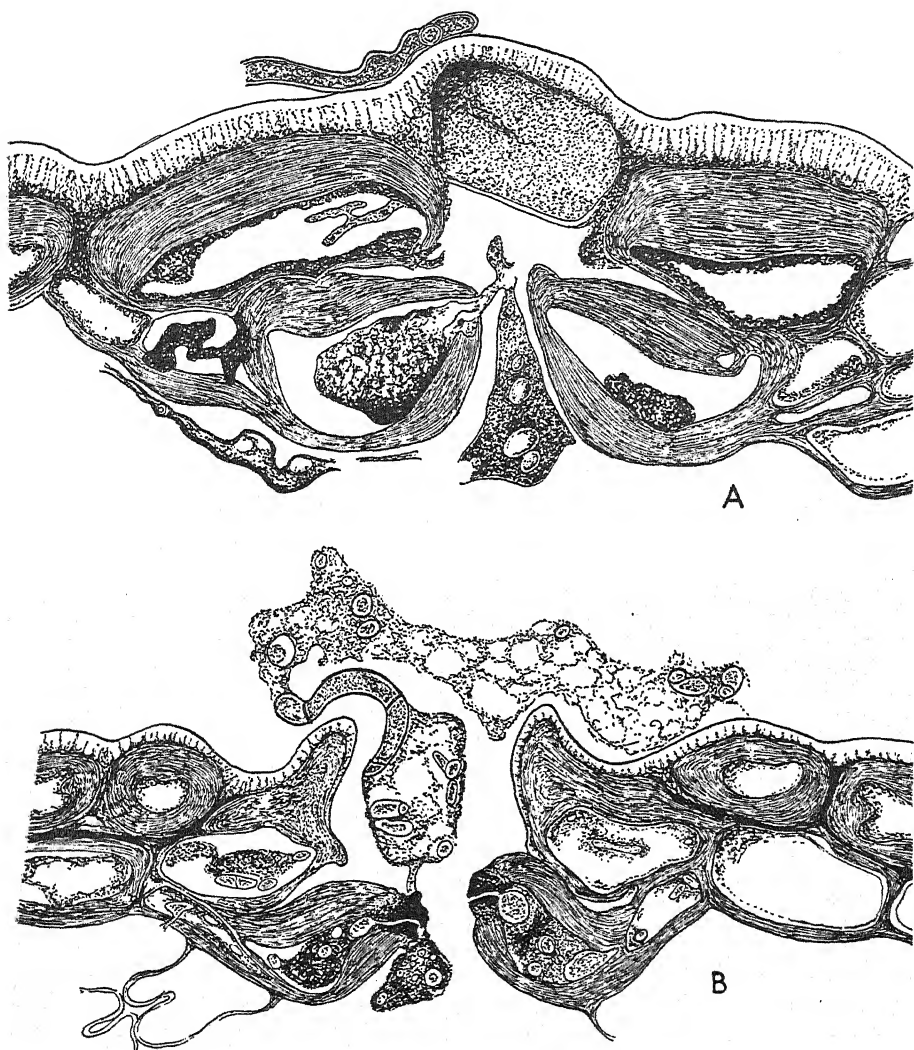


FIG. 3. A. Transverse section of a stoma. Note coarse mycelium on leaf surface; the invading hypha has sent a branch into a guard cell, continuing its growth into a vesicle from which branches arise and the whole is densely black—the 'scleroté'; coarse mycelium on the left. $\times 2200$. B. The invading hypha within stomatal pit surrounded by expanded gelatinous sheath; invasion of both guard cells through fine pores; portion of scleroté as above. $\times 1200$.

extends along the radial walls, and there is no doubt that this dark coloured substance, which is so prominent in the preparations, consists largely of altered host-cellulose impregnated with fungal pigment. The middle

lamella in the radial walls seems to be affected in this manner (Fig. 4) and the hyphae are now capable of digesting their way through the matrix, thus gaining access to the tissues of the pericycle. The selection of the middle lamella as the point of attack would appear to be made because the broad

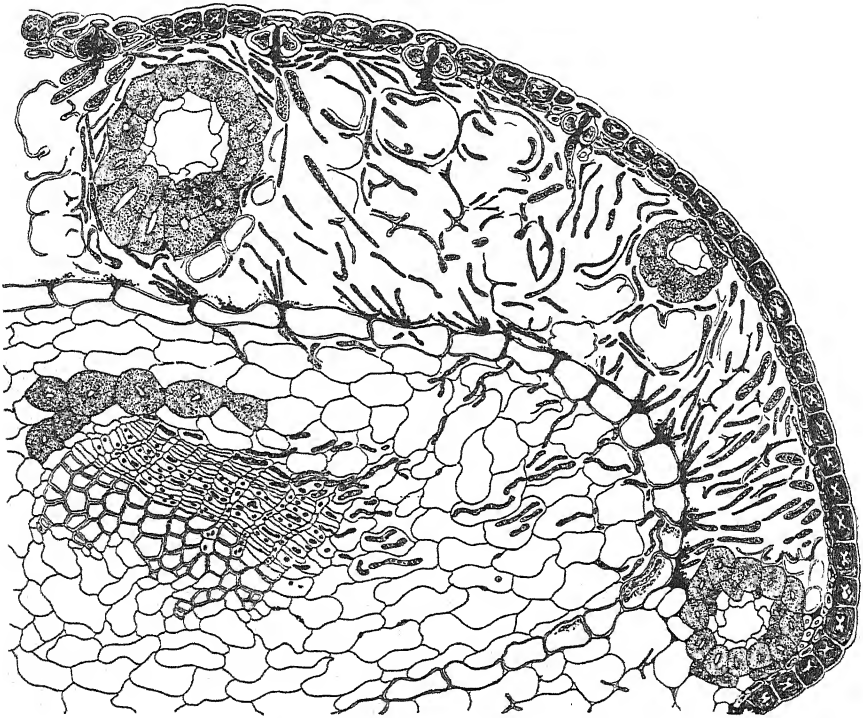


FIG. 4. Portion of transverse section of leaf showing mycelium in the disintegrated mesophyll. Note the black substance in the radial walls of the endodermis and penetration of the pericyclic tissue, the fungus finally occupying the phloem. The xylem and pitted elements of the transfusion tissue are free of fungus (diagrammatic).

casparian strip seems to form an impenetrable barrier to the fungus; in rare instances, however, a few hyphae can be seen to pass through its perforations into the adjacent endodermal cell. It is peculiar that there is not more penetration at the tangential walls of the endodermis, and when this does occur the hypha coils within the cell before it finds exit by attacking the opposite wall. The mycelium now within the stele takes the shortest route to the phloem (Figs. 4, 5 C), and unlike its inroad into the mesophyll, its mode of attack now is by penetration of the cell-walls and only a few instances are seen where the fungus is intercellular. It is very significant that very few xylem tracheids of the vascular bundles and never any of the pitted cells of the transfusion tissue are occupied by the fungus, the cells parasitized being those of the phloem and the living parenchyma,

including those of the medullary rays. Tubeuf (25) and Langner (18) have reported that the transpiration current is more active from diseased than from healthy leaves, the reason probably being that the vascular tracts of the leaf are not only practically clear of obstruction (Fig. 4) but that the

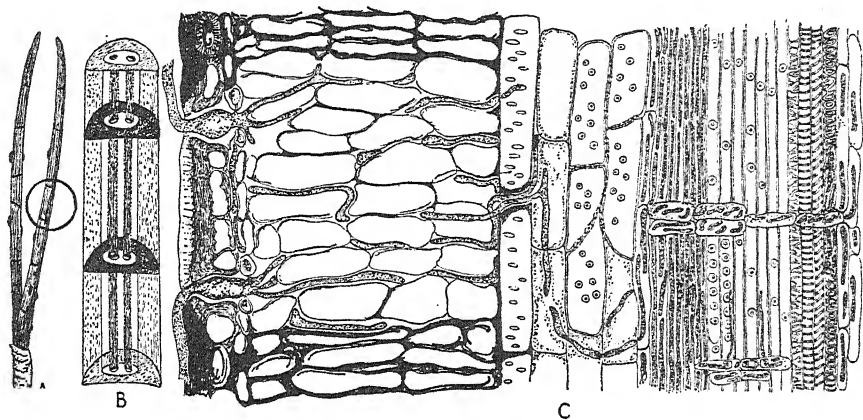


FIG. 5. A. The black rings or diaphragms dividing the leaves into zones. B. Perspective diagram of inset portion of A, showing that the diaphragms are only partial and interrupted by endodermis. C. Portion of a radial leaf section showing the fungal vesicles in the sub-stomatal cavities, the fungus penetrating the guard-cells and the intercellular spaces of the mesophyll; the thickened darker layers are those of the diaphragms; endodermis is invaded at the thickened wall of black deposit; invasion of the living parenchyma of the stele, the phloem and medullary rays. Xylem and pitted transfusion cells sparsely occupied by fungus (diagrammatic).

process may be uncontrolled owing to the crippling of the stomatal guard cells as described above.

Most of the authors mentioned above have observed the existence of narrow black rings which completely encircle the leaves. These arise at varying intervals along the whole length of the needles, first as brown rings which turn black as soon as the pycnidia approach maturity. As shown in Fig. 5 A, B, C, the rings are really partial diaphragms, extending completely across the mesophyll, but stopping at the endodermis. In the longitudinal section (Fig. 5 C) they consist of a few layers of dead mesophyll tissue in which the cell-walls are somewhat thicker, presumably by swelling, and heavily impregnated with black pigment. Against the clearer zones of mesophyll they stand out like plates of stereome tissue, and whilst the membranes of the clear zones are largely disintegrated, those of the diaphragms remain comparatively intact and thickened. When it is considered that the fructifications are laid down surrounded by disintegrated tissue, it is probably a function of the diaphragms to give mechanical support to the superficial layers of the leaf. Further, such a partitioning of the disintegrated tissue into zones may also bring about nutritive changes within them, possibly of the nature of staling, a factor which is frequently concomitant with the formation of fungal fructifications.

It will be of interest to discuss some of these observations on leaf infection so far described. It is borne in mind that the leaves are still on the tree and that during pycnidial development the leaves are not entirely devoid of chlorophyll, but when the apothecial primordia begin to appear the foliage is brown and the black pigmentation of the diaphragms at this time is also very significant. Further, repeated observations have revealed the facts that the pycnidia are usually fully developed before the fungus has penetrated the endodermis, and that the incidence of apothecial development seems to coincide with the establishment of the mycelium in the phloem tissue of the leaf. It is, however, very difficult to be certain on this point as the apothecial initials follow so quickly upon the maturation of the pycnidia. Langner (18) points out that on sterilized dead needles of *Pinus sylvestris* the fungus produced pycnidia but no apothecia, and that on sterilized living needles there was no growth. Wille (26) states that infection in May or June occurs when the leaves are at a period of minimum acidity and that the production of pycnidia is largely dependent on conditions prevailing during the preceding winter.

To complete the writer's observations on the presence of mycelium within the host it will be necessary to mention some divergent views on this point. Tubeuf (25), in his experimentation on infection in the open and under greenhouse conditions, states that mycelium is mostly confined to the needles, adding that the plants die rather from excessive loss of water from the dead needles than from hunger. However, taking five-year-old plants to the greenhouse in October, Tubeuf found that some of the fourth-year leaves became brown and diseased in appearance; the following spring some of these plants developed healthy green leaves, amongst which he saw browned leaves containing the mycelium; he collected them and placing them in a moist chamber obtained, after some weeks, apothecia. Hartig (14) maintains that if diseased seedlings are protected under favourable conditions the plants may survive, but never if mycelium has penetrated the axis, and that if the pith cylinder is browned the plant dies even if its buds appear healthy. Langner (18) is of opinion that fall of the dwarf shoots cannot be attributed to a disorganization of the water balance and that dessication of the branches associated with leaf-fall is a direct consequence of infection. It is the writer's experience that great caution must be exercised that the trees under observation are not simulating the disease through merely faulty water relations so frequently observed after periods of frost or drought. Tubeuf (25) states that the diseased needles are cast off prematurely by the formation of cork layers at the base of the dwarf shoots (Fig. 6 c). This of course is a common feature of the disease, and when the axis adjacent to the absciss layer is examined, no mycelium can be seen in the stem. But the writer has observed that fall of the dwarf shoots can also be brought about from

deposition of the black substance in the region that would normally form the cork barrier (Fig. 6 A). The black substance appears to be as effective in inducing leaf-fall as cork, but with the important difference that the

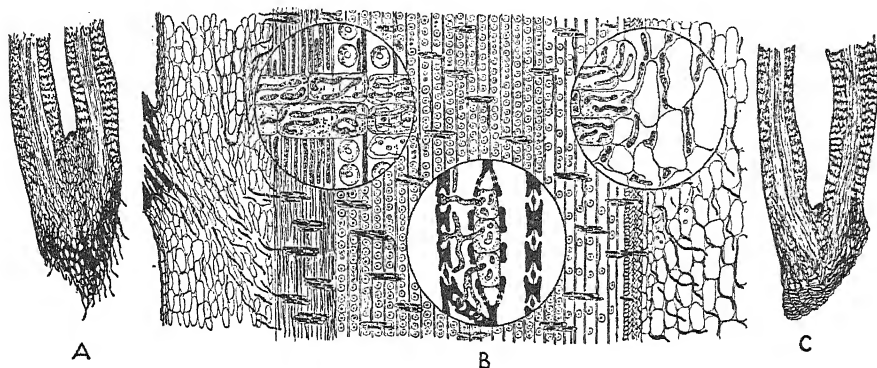


FIG. 6. A. Longitudinal section of basal part of a dwarf shoot infested with fungus and dense deposit of black substance at the base. B. Radial longitudinal section of a young stem invaded by fungus from seat of such a dwarf shoot as A. The fungus traverses cortex, living cells of rays and phloem, the xylem by means of the rays, finally reaching the medulla. Three inset drawings of fungus in rays and phloem; in living cells of a ray shown in tangential section with bordered pits forming barriers to invasion; fungus invading medulla from the rays. C. Longitudinal section of basal part of a dwarf shoot infested with fungus, but with normal cork-layers causing fall of shoot (diagrammatic).

fungus is able to negotiate this matrix, and presumably, this is accomplished before the actual fall of the shoots.

Fig. 6 B, shows a radial section of a young twig cut near the base of the current year axis. The path of the fungus is seen on the left to start from the point of attachment of a dwarf shoot; it proceeds across the cortical tissue to the medullary rays and the phloem which it penetrates, thence by way of the living parenchyma of the rays it traverses the xylem, finally reaching the medulla. It is also noticed that a few attenuated hyphae have entered the xylem tracheids from the medullary rays by means of their simple pits. The bordered pits of the tracheids, however, form an impenetrable barrier to the fungus (Fig. 6 B); a hypha invading a pit immediately pushes the torus in front of it, thus effectually sealing the opposite pore, and not a single case has been seen where a hypha has been able to circumvent the torus to penetrate the pit membrane. The scarcity of mycelium in the wood will perhaps explain why the water relations of the plant and the process of transpiration are not largely interfered with, a conclusion to which reference has already been made in connexion with the distribution of the mycelium within the tissues of the leaf. The two methods described, whereby the tree loses its leaves, with or without the formation of cork, may possibly explain why the presence of mycelium in the axis has not been detected in all cases.

The Fructifications.

Up to this point the writer has employed the term pycnidia for one type of fructification in *Lophodermium* because this is the term that has

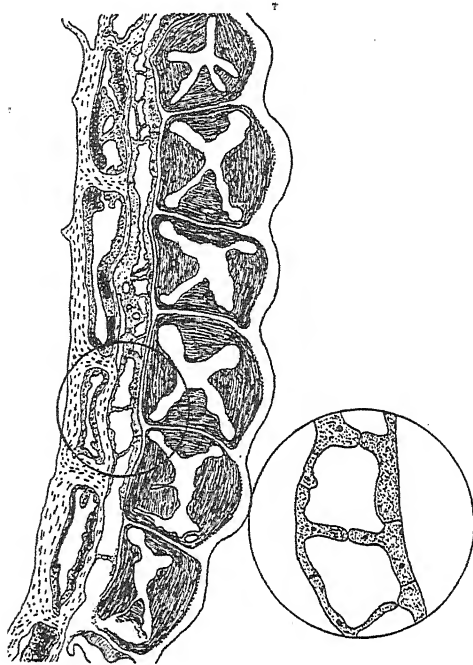


FIG. 7. Transverse section of a portion of leaf about to form a fructification. The mycelium is between the epidermis and hypodermis; in parts it is of the coarse type, and anastomoses are seen between some branches of the finer type. $\times 1200$. Inset, details of hyphal anastomoses.

largely been in use in the literature. Henceforth, for reasons stated below, these organs will be called spermogonia.

The spermogonia and apothecia are laid down between the epidermal and hypodermal layers of the leaf (Fig. 7). At the areas where these organs are to become established the mycelium is of fine texture with some of its branches showing frequent anastomoses. In connexion with the process of roofing of these fructifications certain changes which take place at the epidermis are of importance and lend strong support to conclusions made below that some apothecia become established within spermogonia. Whereas the latter are roofed over in characteristic style, the apothecia show two ways, one, exhibiting the spermogonial method—with later additions—and the other, in far greater preponderance, initiated in a different and distinctive fashion. These methods will be described in connexion with the development and structure of the fructifications. Incidentally,

Tubeuf (25) has described and figured two types of apothecia differing in respect of roof formation as illustrated here.

The Spermogonia (= Leptostroma pinastri Desm.).

The minute black and oval-shaped spermogonia generally appear towards the end of summer, but the time varies according to relative humidity. Tubeuf (25), Hartig (14), and Dufrenoy (8) have also observed that their appearance is correlated with meteorological conditions. Tubeuf (25) and Prillieux (21) have figured the spermogonia and Hartig refers to them by this name, adding that they are functionless, but none of these authors has described them in detail. As previously stated, the spermogonia are laid down at any point within the leaf between epidermis and hypodermis; they are therefore, according to Kempton (16), of the nature of acervuli.

As seen in transverse sections some spermogonia are roofed over by some half-dozen or more epidermal cells complete with their inner lamellose thickenings intact (Figs. 8, 9), whereas in others one or two of these cells may be missing either at the centre or towards one or both margins of the roof. These gaps, which extend nearly the whole length of the spermogonial roof, will eventually be the seats of dehiscence. As yet there are no actual gaps for it is only the sclerosed internal lamellae of the epidermal cells which are missing. Removal of the lamellae is presumably accomplished by enzymic action dissolving away the lower half only of the primary epidermal wall, the sclerosed thickenings then falling to the floor of the future spermogonium, appearing there as small sclereides resting on the hypodermis. The mycelium which is already present between the superficial tissues now divides actively, pushing up a small area of the epidermis and proceeding to form a plectenchyma of some three or four layers of oval cells over the hypodermis. With increased curvature of the epidermal roof the upper cells of the basal tissue, which may now be called the hypothecium, proceed to form a great array of elongated and erect spermatiphores (Fig. 10).

A remarkable feature of development at this point is the clearness of the nuclei within the bases of the spermatiphores, and though minute in size they are much better organized than those of the vegetative mycelium in which they are exceedingly small and homogeneously stained. Nuclear division, though undetected, presumably takes place in the base of the spermatiphore and a spindle-shaped nucleus, frequently two, can be seen distinctly in the narrow hypha. Spermatia in enormous numbers are abstracted from the attenuated tips, several from each hypha frequently remaining attached in short chains. They are very small, varying from 4 to $8 \mu \times 0.5 \mu$, and bacilliform in shape; fixed and stained in the same way as the ascospores they are seen to contain a well-marked nucleus surrounded

by scanty cytoplasm (Fig. 10). They are embedded in a clear mucilaginous matrix, and where the epidermis is broken through at their maturity it probably does so by the swelling of this substance which then exudes at

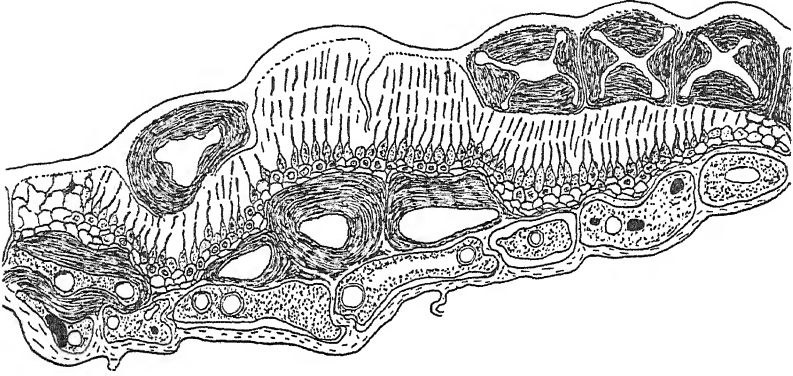


FIG. 8. Transverse section of a small spermatogonium. $\times 1200$.

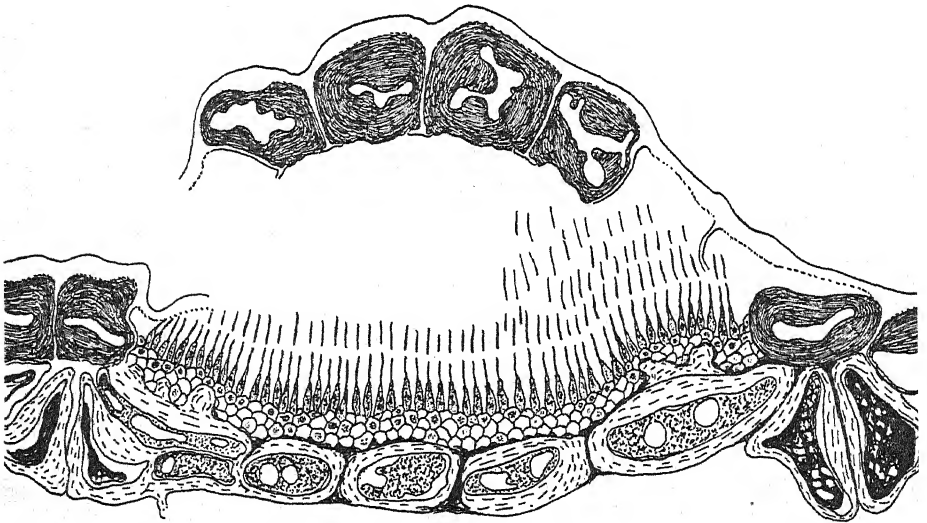


FIG. 9. A mature spermatogonium with marginal dehiscence. $\times 1200$.

the fissures, carrying with it a dense mass of the minute cells. They have not been seen to undergo any growth or changes on artificial media.

In some spermatogonia, not in all, certain vertical hyphae interspersed among the spermatophores are seen to differ from them in shape and nuclear contents. Whereas the spermatial hyphae are uniformly narrow with pointed tips, those now described are broader with smooth dilated terminations. Even when a spermatophore is about to form a spermatium

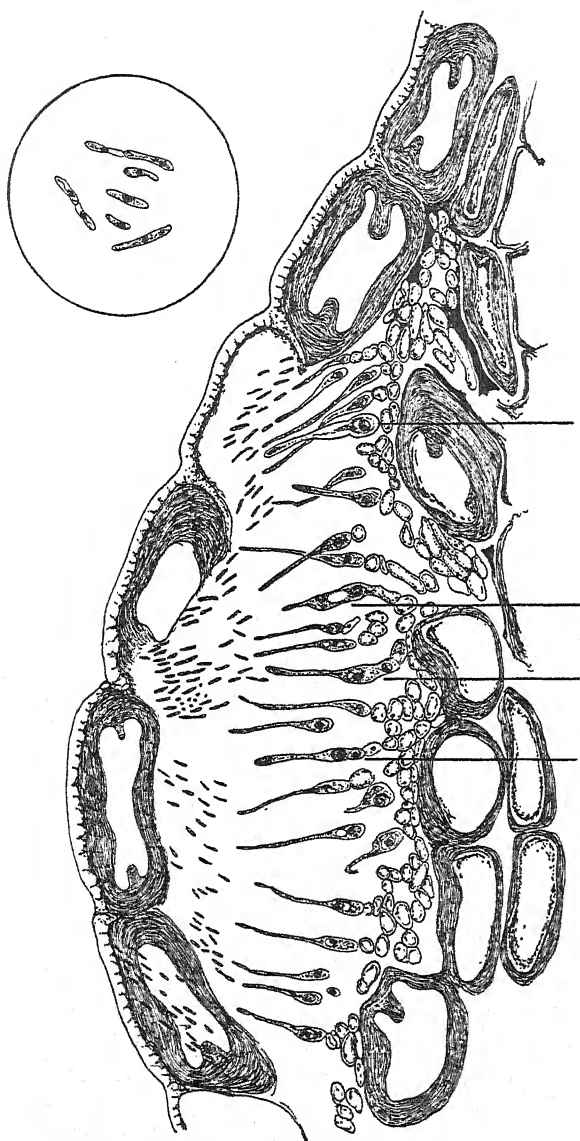


FIG. 10. Portion of a sporogonium in transverse section. The spermatophores are very narrow and pointed at the tips. The four vertical lines indicate the presence of oogonal cells bearing trichogynes. $\times 1100$. Inset: Spermatia. $\times 1650$. (Numerous spermatophores are omitted in order to show up contrast between the elements.)

its elongating apex with the constriction immediately below is quite different in contour from that of one of these special vertical hyphae. Moreover, the basal part of this type of hypha is oval in shape and larger than the base of a spermatophore in which it is truncated. Again, the

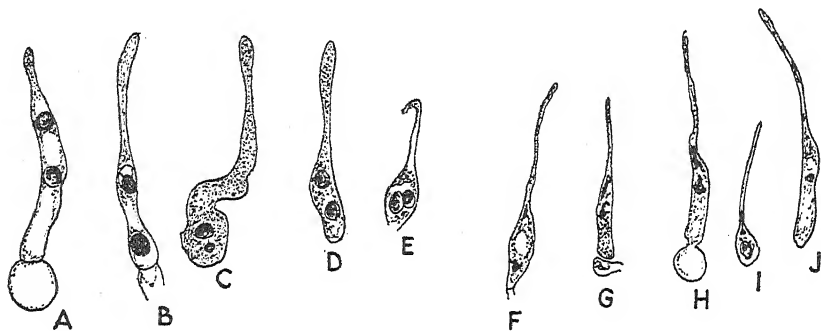


FIG. 11. A-E. Oogonial cells all binucleate. In E, fusion appears to occur and the trichogyne is withering. F-J. Spermatophores. $\times 1730$.

nuclei, single or in pairs are even better organized than those of the spermatial hyphae (Fig. 11). In a series of sections running the whole extent of a spermogonium the specialized hyphae are relatively few in number. They are clearly not of the nature of buffer cells described by Andrus (2) and Allen (1) as occurring in the rust fungi investigated by them. On the contrary they are strongly reminiscent of the young procarys of members of the Rhodophyceae, the erect club-shaped hypha being a trichogyne and the dilated base a carpogonium. In view of subsequent development described below, the writer puts forward the interpretation that a vertical hypha and its expanded base may perhaps constitute the trichogynous and oogonial parts of a sexual apparatus. Higgins (15) describes a similar structure (but there are differences in sequence of development) in the history of *Sphaerella Bolleana*, a type in which the perithecia arise below the spermogonia. He says, 'the trichogyne is erect and there is no distinct line of demarcation between the enlarged basal portion and the trichogyne. There are two comparatively large nuclei in the basal portion . . .'. He adds that the question of the origin of the nuclei and their fusion must remain unsettled.

In the present investigation the trichogynes are in a position to make contact with the spermatia and it is probable that a process of fertilization takes place similar to that described in Lichens and certain members of the Rhodophyceae and as observed also by Andrus (2) and Allen (1) in types of the Uredineae described by them. Owing to the density of the spermatophores and spermatia, copulation with trichogynes and transference of nuclei have not been seen here. It is possible for such a process of fertilization to take place whether a spermogonium is open or closed, but in the

former case there would be the advantage of the transference of spermatia in raindrops (or by insect agency) from one spermogonium to another. Instances have not been seen of trichogynes emerging through the spermogonial fissures nor does the need arise, since foreign spermatia arrested by

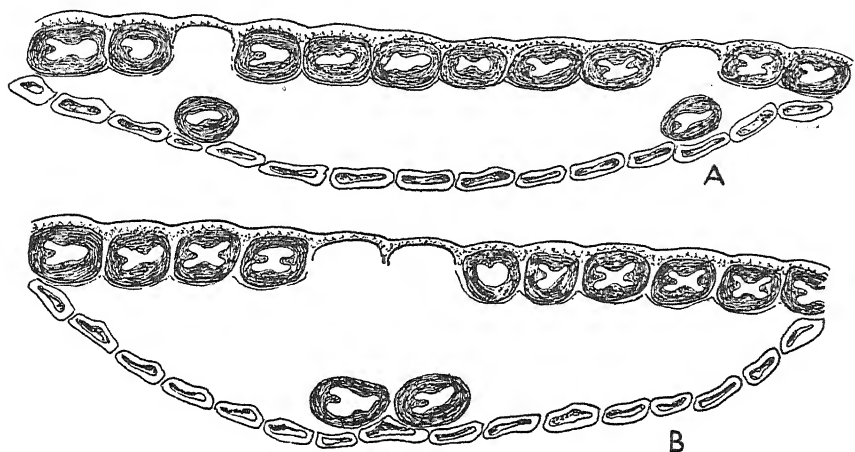


FIG. 12. A, B. Transverse section of young apothecia accommodated within spermogonia. The epidermal lamellae are intact except for two marginal rows in A, and two median rows in B. $\times 530$. (Diagrammatic.)

the mucilage could conceivably be drawn into the spermogonium when this material shrinks under drier conditions.

Some spermogonia, namely, those which harbour the trichogynous oogonial cells may become converted into apothecia. Other spermogonia in which there is no evidence of the presence of trichogynous cells seem to form spermatia for quite long periods, but they eventually dry, out becoming filled with a secretion of black substance in which are often embedded dense masses of spermatia. Incidentally apothecia, in greater numbers, can arise independently of spermogonia. The question of the origin of the trichogynous cells is discussed below.

The Apothecia.

The apothecia are very small, about 0.5×1 mm. (Fig. 1), elliptical and conchoidal in shape, and densely black in colour. They appear on the leaves at any points between the black diaphragms, very frequently along the margins, and more on the abaxial than the adaxial leaf surface. For the most part they arise singly and their occurrence in pairs or small stromata is very rare. They can be seen in some of the zones along with spermogonia; other zones are exclusively occupied by them. When it is borne in mind that apothecial initials continue to be laid down as long as the leaves

are on the tree it is often very difficult to distinguish them from spermogonia except when the latter are open.

In the same way as the spermogonia the apothecia arise between the epidermal and hypodermal layers. If a spermogonial cavity is to be utilized

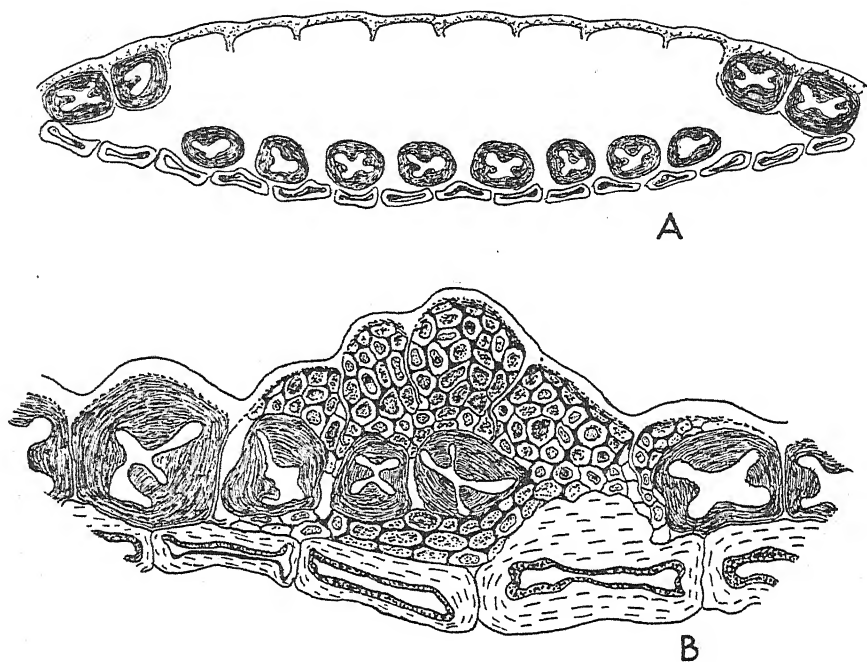


FIG. 13. A. Transverse section, showing the limits of an apothecium, formed independently of a spermogonium, and removal of epidermal lamellae on a wide scale. $\times 530$. B. Early stage of apothecium development; mycelium is filling in the spaces left after removal of lamellae; hypodermis at the base. $\times 1200$.

there is no further removal of epidermal lamellae, and but for this distinctive feature it would be impossible to say what apothecia, at maturity, were preceded by spermogonia or not (Fig. 12). When the change over does take place development is so rapid that by the time the hypothecial cells are pushing in new hyphae it is impossible to recognize any defunct spermatophores, nor are there any signs of spermatia except in few instances where they can be seen arrested against parts of the blackened roof; their identity within deposits of black substance is difficult, for an early stage preparatory to apothecial accommodation is the restoration of the spermogonial roof whereby the fissures become closed with deposits of black substance.

For the building of apothecia which arise independently of spermogonia the removal of epidermal lamellae takes place on a so much larger scale than in the spermogonia as to suggest that the comparatively fewer apothecia

which are covered over by the epidermis in the same way as the spermogonia have previously harboured spermogonia.

Within a prescribed area, in conformity with the elliptic shape of the future apothecium, removal of the sclerosed lamellae occurs along a median

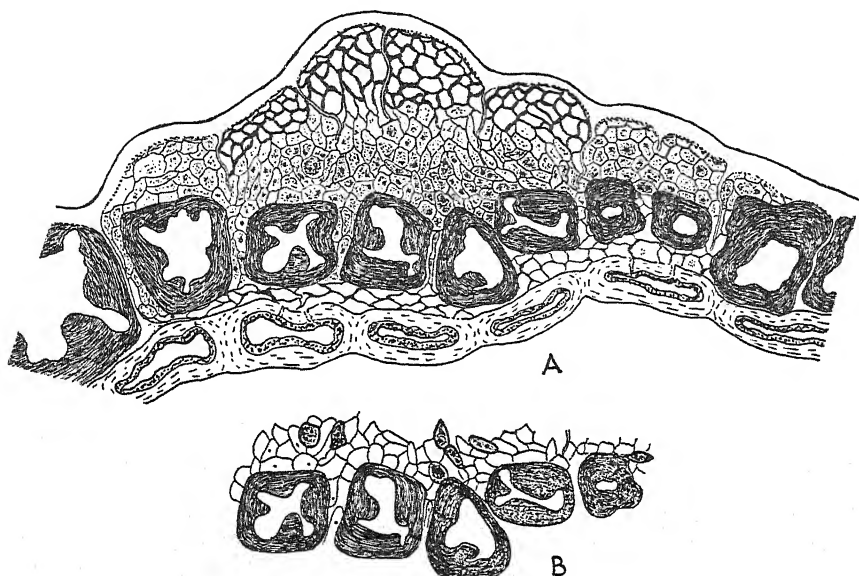


FIG. 14. A. Further early stage in apothecium development; the mycelium shows differentiation into upper roof-building elements and a basal hypothecium. Note in the latter towards the centre a number of cells with darker contents, some uninucleate, others binucleate. B. The denser cells picked out from the above drawing and contents of remaining tissue omitted. These cells are described as ascogonial cells. $\times 900$.

ridge nearly the whole length, mostly at the centre and decreasing gradually towards the extremities. The process also frequently involves the removal of several pairs of guard cells which are often seen along with the cell-lamellae resting on the hypodermis. The mycelium within now proceeds to grow apace, rapidly filling in the gaps left in the roof by the lamellae with a somewhat loosely woven plectenchyma and establishing itself at the base to form the hypothecial tissue, these features being identical in both types of apothecia whether preceded by spermogonia or not. As previously stated, Tubeuf (25) gives figures of apothecia with the epidermal covering complete as well as others in which the lamellose thickenings are missing, but makes no distinction as to their derivation.

The process of roof-building or thickening is an early feature in development, and it is very significant that there should appear in the hypothecium at such an early stage a number of dark-stained cells in relatively scattered positions within this tissue (Fig. 14). Some of the

deeply stained cells are uninucleate, others binucleate, and their subsequent history leaves no doubt that they are the ascogonial initials.

The appearance of these cells at such an early phase may be accounted for in the case of those apothecia established within spermogonia if the

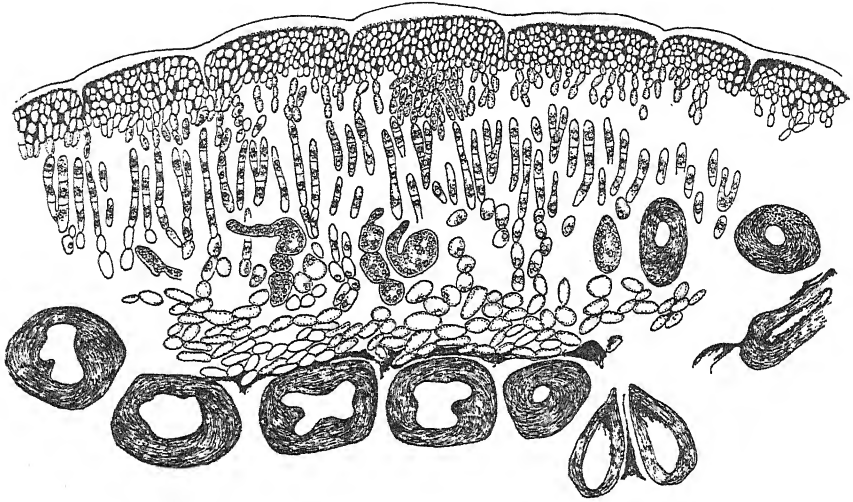


FIG. 15. Transverse section of a young apothecium showing prominence of ascogonial cells, now multinucleate, and some putting forth ascogenous hyphae; the vertical hyphae are roof-building elements; note below the central epidermal cell a group of oblique hyphae to form the periphyses; epidermal lamellae and guard-cells at base. $\times 430$.

observations here are correct and that they can be identified as the trichogynous oogonial cells described above. But what of their origin in other apothecia, for they appear in them at the same stage of general development? In the absence of evidence obtained from artificial culture and the difficulty of recognizing the dark cells at an earlier stage than here depicted it is very difficult to trace these cells to their origin. Langner's (18) discovery, however, that the fungus possesses several strains and is probably heterothallic, together with the observations set forth here showing evidence of multiple spore-infection and early anastomoses in the mycelium, are strongly in favour of the view that the dark stained cells may arise from the fusion of certain cells derived from the intermingling of mycelia of opposite strains. Such points of fusion or of nuclear fusion within the cells have however not been detected here. This question will be discussed again, and at this juncture the further history of the ascogonial cells will be described parallel with that of general apothecial development. The apothecia proceed to increase their accommodation as soon as the ascogonial cells are established. More and more epidermis is lifted up from the hypodermis, but there is no further dislodgement of internal lamellae. With increased expansion the mycelium proceeds to add to the thickness of the roof, not only within the

median ridge, already partly covered, but the whole extent of the raised epidermis. At this point it becomes apparent that there is a difference in the way the elements are being laid down at the median ridge, and elsewhere in the roof (Fig. 15). In a transverse view of the ridge the elements

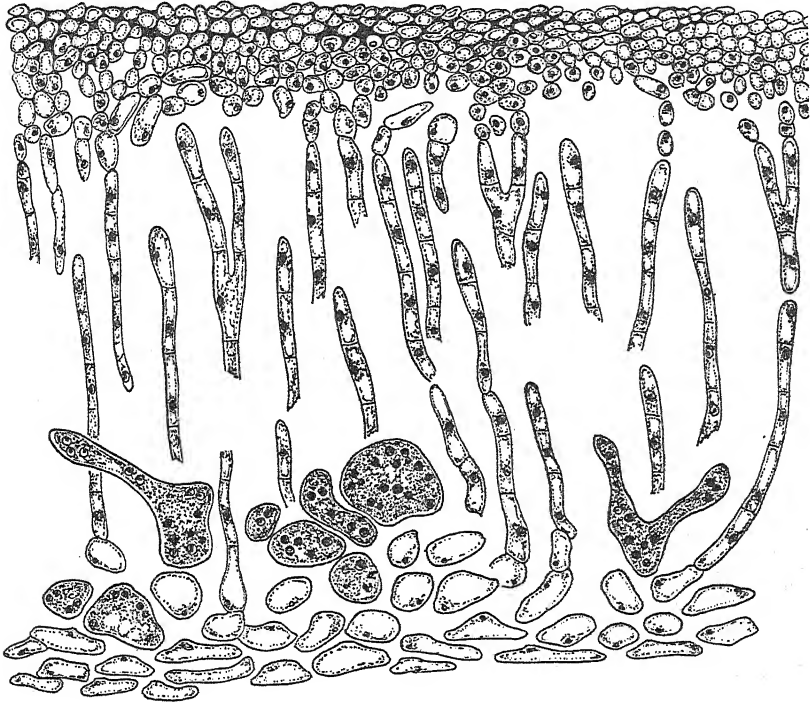


FIG. 16. Portion of transverse section of young apothecium showing formation of ascogenous hyphae from the multinucleate ascogonial cells which occur singly or in short coils. The vertical hyphae are mostly those of roof-building, but true paraphyses are just arising from the hypothecium. $\times 1605$.

are disposed in two groups facing each other, the hyphae being arranged lengthwise and interlocking somewhat loosely with each other in an upwardly oblique manner (Fig. 20). They are described and figured by Tubeuf (25) who calls them 'papillae' and Prillieux (21) describes similar features in *Lophodermium macrosporum*. These structures are not merely papillae in the sense that they consist of thickened cuticle, but are hyphal cells with living contents. Their mode of origin, position, and orientation show that they are true periphyses arising in the same way as those of members of the Pyrenomycetes. Their function is concerned with dehiscence of the apothecium, and further details of their structure will be resumed below. The process of roof-thickening goes on for a considerable time, and as more and more cells are added from below the older layers at the periphery lose their contents and become firmly cemented together. As

roof-thickening goes on hand-in-hand with the development of the apothecial tissues it follows that towards its completion the superficial cells of the hypothecium which were devoted to it will in parts maintain their connexion with the last elements laid down in the roof (Fig. 16), thus giving

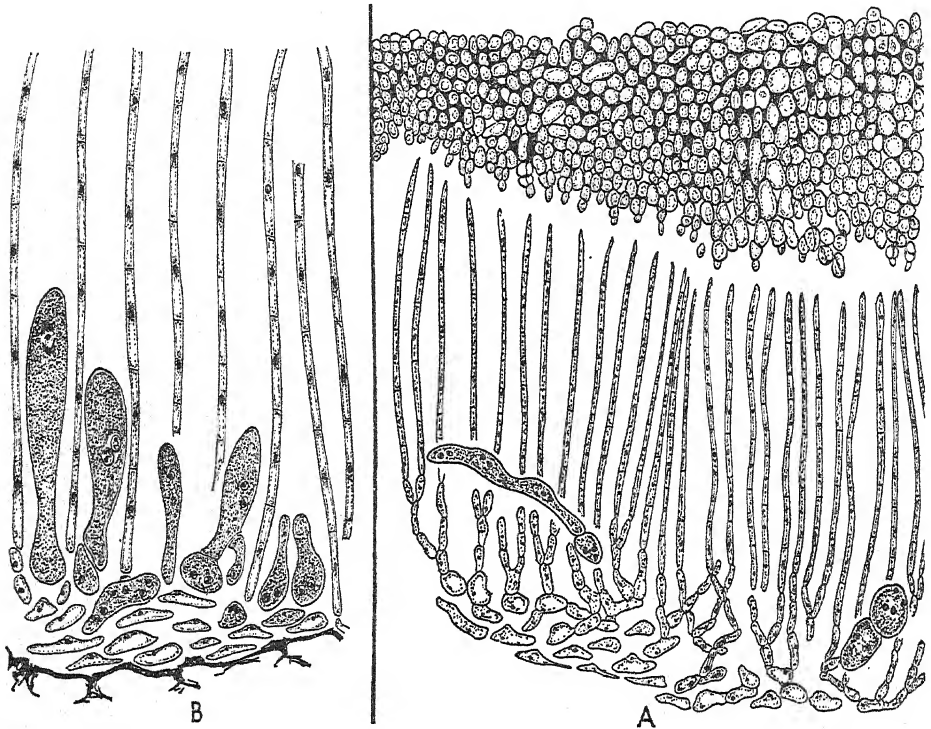


FIG. 17. A. Transverse section of apothecium forming ascogenous hyphae; the paraphyses are prominent, completely dwarfing any roof-building elements left at the base. 1100. B. Portion of hymenium showing formation of asci, some with intervention of a crozier, others direct from the ascogenous hyphae. $\times 1650$.

the appearance of vertical trabeculae; in other parts where the continuity is not maintained small chains of cells will hang from the roof or remain erect on the hypothecium. These details are emphasized at this point in order to show that these more or less vertical hyphae are definitely not paraphyses but remnants of roof-building hyphae. The paraphyses are a later development appearing along with the ascogenous hyphae or later (Fig. 17 A).

The Ascogonial Cells.

With the process of roof-building well advanced and the periphyses formed (Fig. 15) the dark-stained ascogonial cells become very prominent, and meanwhile they have increased their nuclear content considerably, and cell-division has presumably taken place, for short chains or coils of three or

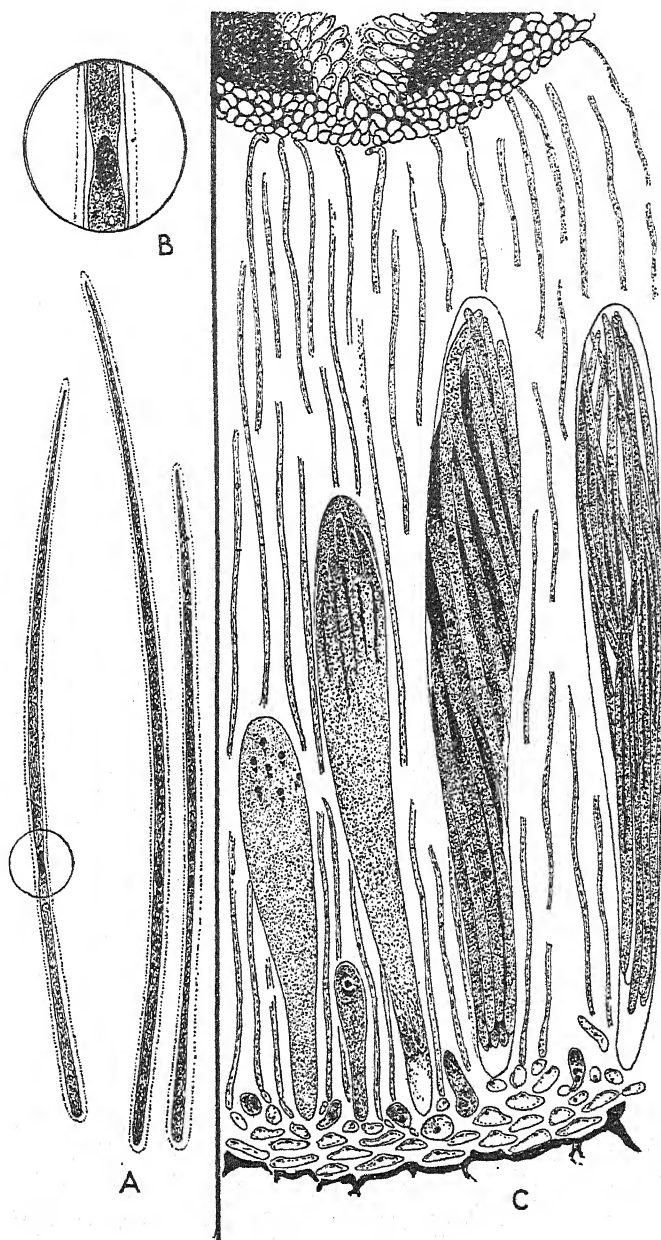


FIG. 18. A. Mature ascospores (stained Heidenhain's iron-alum haematoxylin), showing the single nucleus and highly granular contents. $\times 1000$. B. The nucleus in A; note thicker wall. C. Portion of a transverse section of nearly ripe apothecium with asci at various stages of maturity; note cleavage of cytoplasm accompanying spore-delimitation. The ascospores on the right are exhibiting torsion; attenuated paraphyses bent at the tips from pressure of roof. $\times 900$.

more are now seen (Fig. 15). It is still very difficult to say whether the minute nuclei occur isolated or in pairs, and no definite statement can be made that fusion takes place. The ascogenous cells have not been seen to communicate by pits or by absorption of their intervening walls.

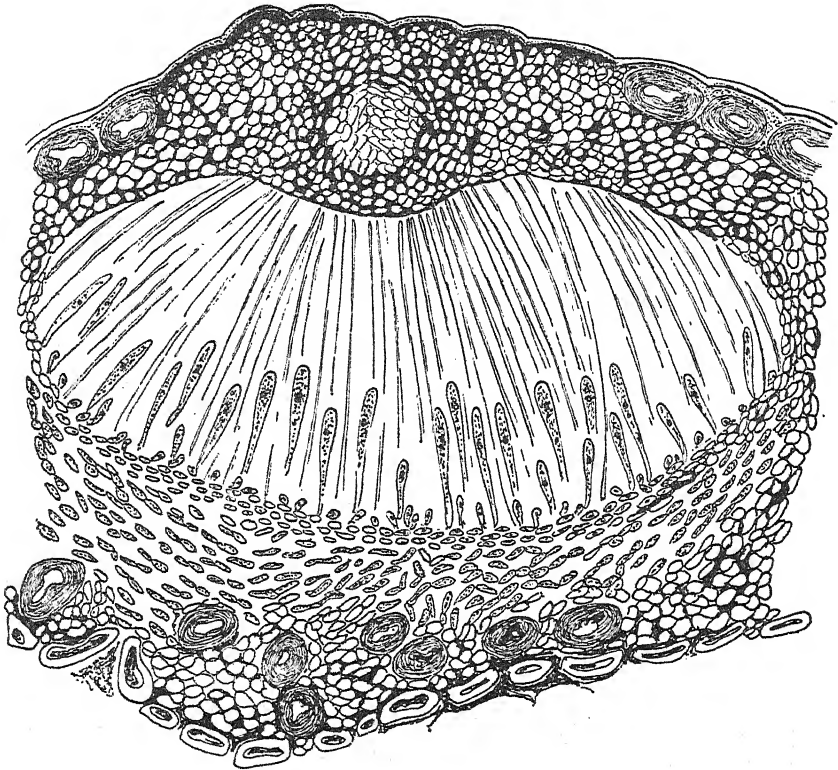


FIG. 19. Transverse section of a typical apothecium at time of active formation of asci. Note the periphyses within the median ridge of the roof; epidermal lamellae and guard-cells below the hypothecium. $\times 530$.

It is noteworthy that when roof-building is complete and the ascogenous cells are about to produce ascogenous hyphae, fall of the dwarf shoots generally takes place, but these events are not constant, for ascogenous hyphae seem to be initiated over comparatively long periods. The completed roof, however, is a fairly constant sign of approaching leaf-fall.

With increased expansion in the apothecium the ascogenous cells are seen to put forth ascogenous hyphae approximating in diameter almost the cells themselves, and seemingly any cell of a group is capable of producing them (Fig. 16). At this time, or later, the formation of paraphyses from the superficial cells of the hypothecium is very active; they are very narrow, consisting of elongated uninuclear cells. Previous to apothecial dehiscence

they are straight (Fig. 17 A) but become bent at their tips when the roof presses on them just before dehiscence takes place (Fig. 20 C, D).

The nuclei migrate into the ascogenous hyphae in pairs, and for their reception the hyphae may or may not assume the crozier habit, and with or

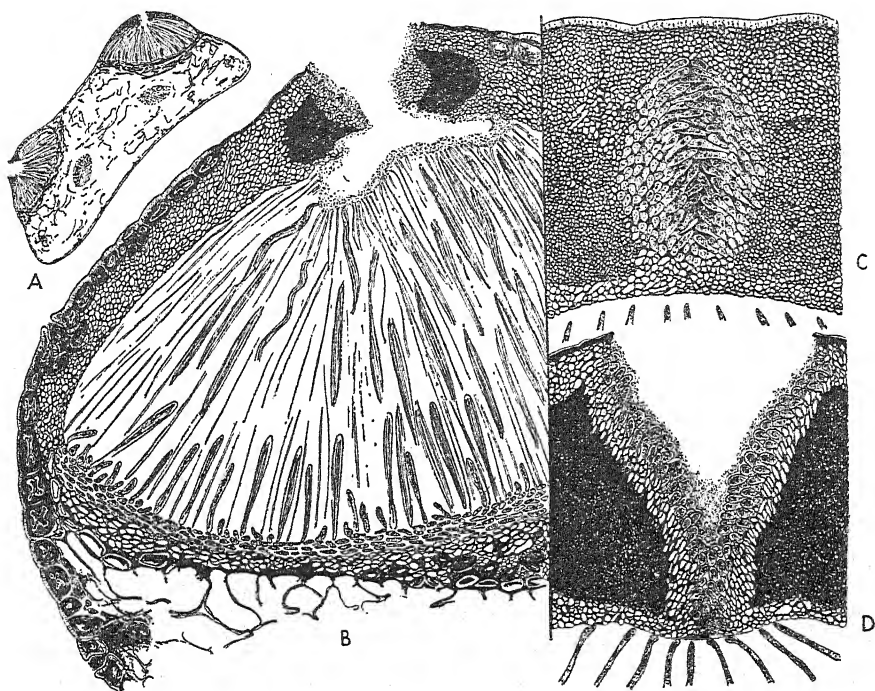


FIG. 20. A. Transverse section of a leaf with two mature apothecia (diag.). B. Portion of a mature apothecium at spore discharge; note the dehiscent fissure with periphyses and mucilage which is continued into the cavity. $\times 173$. C. Transverse section of the median ridge of a roof showing the interlocking periphyses and mucilage within; tips of paraphyses below are straight. D. The fissure of dehiscence; exposed periphyses and mucilage; note the densely black consolidating tissue behind the periphyses; tips of paraphyses bent. $\times 433$.

without the attendant cross-walls, delimiting the usual stalk and penultimate cells (Fig. 17 B). Whereas the structure of the nuclei in the ascogenous hyphae is undifferentiated that of a fusing pair within the young ascus shows high organization. Although close study has been made of cytological details in the behaviour of the nuclei at this and subsequent stages, no conclusions can be drawn owing to the minute size of the chromosomes.

The eight nuclei are all utilized in spore formation, and this is accomplished by cleavage and vacuolation of the cytoplasm. The young ascospores show an elongated mass of chromatin which as they ripen tends to aggregate towards the centre. In the fully matured asci the bundle of eight spores frequently shows a distinct torsion, an adaptation probably for facilitating their discharge from the apothecia. There are no signs of

apical pores or caps in the mature asci, and previous to spore discharge the walls of the asci gelatinize completely (Fig. 18).

Dehiscence of the Apothecia.

It has been seen how provision for the opening of the apothecia has been made at a comparatively early stage of development (Fig. 19). At maturity the roof is considerably thicker within its median ridge than along the flanks, and with the absorption of moisture, so essential to dehiscence, it is now seen that the loosely interlocking periphyses are furnished with very prominent and swollen tips (Fig. 20 C). With increased absorption the tips become partially mucilaginous so that considerable pressure is set up within the enclosed periphysal region, and owing to the configuration of the roof the ultimate effect will be to cause an external split extending almost the whole length of the apothecium, immediately above the periphyses (Fig. 20 D).

The latter now partially exposed appear very moist and of a slight orange colour; with further curvature of the roof the mucilage within the periphysal fissure gets drier, and finally the split extends right through into the apothecial cavity (Fig. 20 B). The filiform ascospores are discharged intermittently in small clouds, and during the intervals the fissure becomes partially closed. The fissural lips are well consolidated, for they are strengthened by dense deposits of black substance appearing like rods of sclerenchyma immediately behind them (Fig. 20 B, D).

General Considerations.

The present investigation has brought forward additional evidence to the already accumulating mass of information on the correlation of sex and nutrition in the fungi (Gwynne-Vaughan) (10). In those types of fungi where spermogonia and apothecia are developed it is common knowledge that the spermogonia appear first. In such a case as now described it would be of interest to determine the nature of the pabulum which suffices for spermogonial development when the infected leaf on the tree has its chlorophyll still evident in parts. Langner succeeded in producing these organs on sterilized dead needles, but not on green ones similarly treated. The needles are still on the tree when the fungus is about to enter on the second phase of its life-history, and it has been seen that when the apothecial primordia are laid down their appearance seems to be correlated with the fungal occupation of the tissues of the phloem in the leaf-bundle. This progressive penetration of the fungus into the stele cannot be explained entirely on the score that the mesophyll has already been impoverished by spermogonial development, for Langner (18) failed to cultivate the apothecia either on dead or living sterilized leaves. Moreover, the appearance of

ascogonial cells is an early incident in the young apothecia, and as soon as they are established leaf-fall is not long delayed. It seems reasonable to state, therefore, that even at this early stage of apothecial development the conditions for complete growth have already been established before the dwarf shoots are cast off. When the leaves are on the ground successful hibernation demands only that they are well covered under moist conditions in their own carpet of leaf-straw. The factor of seasonal change is probably one of minor importance, but it is possible that a chemical factor enters here when partial decay of the infected needles has altered their reaction to the extent of stimulating the apothecia to full fruition. In artificial culture these questions cannot be solved from considerations of carbon-nitrogen relations or of hydrogen-ion concentrations alone, for there are other subtle factors operative, the most important perhaps being the right selection of complementary strains which are receptive only under a very delicate balance of the constituents of the medium and its reaction.

There is abundant evidence that multiple infection by ascospores takes place in the leaf, and though *Lophodermium pinastri* has proved refractory in culture Langner's (18) discovery of several strains and of probable heterothallism is very illuminating in the interpretation of the details set forth in the present work. It is conceivable that an ascospore of one sexual strain would produce mycelium forming only spermogonia; but when mated in multiple infection with mycelium derived from ascospores of opposite sexual strain, there would result from the intermingling of the two mycelia a spermogonial organ in which the trichogynous oogonial cells would develop side by side with spermatial hyphae resulting in an apothecium being established under true sexual conditions. Though the spermogonia are only a fleeting phase in the life-history, far from being functionless organs, they may play an important part, not only in sexual reproduction and rejuvenescence of the parasite, but provide for nuclear distribution on a wide scale. Whether after the disappearance of spermogonia from the life-cycle the copulating mycelia still remain functional for the production of apothecia at other areas in the leaf, or whether these originate from fresh infections, could not be determined, but in the absence of evidence from artificial culture, it is possible that, as in the present case, when the majority of apothecia arise *de novo*, they do so from the intermingling of mycelia introduced after multiple infection from ascospores possibly of opposite sexual strains. These conclusions are supported by the work of Dodge (6, 7) on the ascomycetous genus *Neurospora*; this author states that by a proper selection of spores on the basis of their size, monosporous mycelia could be obtained which would produce only conidia when grown separately, but which when properly mated would also produce ascocarps. The fact that apothecial stages in several types are only produced when monosporous mycelia are mated with their reciprocal

haplonts is now well established, this type of segregation being referred to as heterothallism. Further, the interesting account given by Thaxter (24) in his monograph of the Laboulbeniales states that the various species possess two sorts of spores usually somewhat different in size; the male spore is frequently somewhat smaller than the female; they are discharged from the asci in pairs and become attached to the host side by side. In *Lophodermium* it is possible that the interweaving mycelia remain haploid until the appearance of the dark-stained ascogonial cells, but whether nuclear fusion takes place in them could not be determined.

The systematic position of the genus *Lophodermium* from the results presented here seems to be further established in the Hysteriales. This group is admittedly on the border-line between the Pyrenomycetes and Discomycetes and the only disturbing factor (along perhaps with the narrow elongated fissure at dehiscence of the apothecium) which inclines towards its affinity with the former class is the presence of true periphyses. But this is a feature of minor importance when it is considered that the main characteristics described here for apothecial development are those which follow on typically discomycetous lines. In most investigated types of perithecial structure the development and ramifications of the ascogenous hyphae are dependent on the partial deliquescence of a zone of tissue within the hypothecium; in an expanded hypothecium as found in *Lophodermium* the sequence of development is strictly that of a discomycete and there is no granulation of any part of its hypothecium during the extension of the ascogenous hyphae. Gäumann (9) suggests the term hysterothecium for this type of narrowly opening ascocarp; there does not seem to be any justification for its introduction, for the term could be applied also to the compound ascocarps of some members of the Phacidiales which begin their dehiscence by narrow slits but become wide open at maturity. On the basis of morphology, in particular that of the ascocarp covering, and of general development of the apothecium, most of the evidence set forth in this paper is favourable to the retention of *Lophodermium* in the family Hypodermataceae of the group Hysteriales in the Discomycetes (11).

SUMMARY.

1. *L. pinastri* is a parasitic fungus causing premature fall of the dwarf shoots of pine.
2. Spermatogonia and apothecia are formed between the epidermal and hypodermal layers of the leaf.
3. Spermatogonia are fully developed on attached leaves before the mesophyll is impoverished.
4. Apothecia are also initiated on attached leaves, there being some evidence indicating that they are formed after the fungus has also pene-

trated the endodermis to occupy the phloem of the leaf-bundle; the water tracts are only sparsely occupied.

5. The spermogonia are possibly functional, their spermatia being considered as male cells.

6. Some spermogonia, in addition to spermatial hyphae, contain structures resembling the simpler carpogonia of the Rhodophyceae and may be interpreted as oogonia bearing trichogynes. Fertilization has not been detected.

7. Some spermogonial cavities may later accommodate apothecia; other spermogonia become defunct.

8. Apothecia arising independently of spermogonia are deemed to develop from intermingling of mycelia, possibly of opposite sexual strains.

9. Apothecia which follow spermogonia and those formed independently are recognized from the distinctive character of the roof-covering.

10. The process of roof formation is described; true periphyses are formed.

11. Development of ascogenous hyphae and spore formation are described.

12. Mode of dehiscence of the apothecia is given.

13. Germination of the spores is described.

14. On basis of morphology and development the systematic position of *Lophodermium pinastri* in the Hypodermataceae-Hysteriales-Discomycetes is retained.

The writer wishes to thank Professor J. S. Boyce for sending pressed material of various species of pines attacked by this parasite. He is also under deep obligation to Dr. M. C. Calder for her great help in the prosecution of this work. Thanks are due to Professor Walton for his interest in the work and his kindness in reading through the manuscript for press, and also to the Executive Committee of the Carnegie Trust for a grant towards the cost of the illustrations of this paper.

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Some Experiments on the Effects of Animal Hormones on Plants.

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With Plate XV and one Figure in the Text.

INTRODUCTION.

THE satisfactory experimental demonstration of the specificity of the action of the animal hormones in plants would be a valuable contribution to our knowledge of the parallelism between the physiological processes of plant and animal protoplasm. Various workers have from time to time examined some of the effects of animal hormones on plants, but the literature is in the main scanty, and such as there is somewhat contradictory. This lack of unanimity among the workers in this field is comprehensible, when one realizes the difficulties which attend the extraction and purification of the hormones which are at present available.

One of the earliest workers in this field was Budington (8 and 9), who studied the action of an extract of thyroid gland on the roots of *Allium* and of *Narcissus*. He found that the extract apparently had a stimulatory effect on the growth of the roots. Similar results were obtained by Niethammer (34), using a similar extract with germinating seeds of cereals and with dormant shoots of *Syringa* and *Aesculus*. Eyster and Ellis (15) found that low concentrations of insulin increased the growth of the roots and of the shoots of Maize seedlings, while higher concentrations had an inhibitory effect. Rebello (38), using hyacinths as his test plants, found stimulatory effects with extracts of suprarenal and of pituitary glands while the freshly grated substance of these glands had an inhibitory effect. His results with the thyroid extract are contradictory to those of Scaglia (40). According to this author, thyroid extracts have an inhibitory effect on the increase in the total weight of the plant, the inhibition being increased

with increasing concentration, while they increase the growth rate and tissue differentiation. Marked differences were found in the effects of different extracts of the same gland. De Gaetani (16), using *Lemna minor* plants, found in the extracts of the suprarenal cortex a powerful stimulant of the development of the sex organs in these plants, while the growth in size was inhibited. Occhipinti (35), using *Azolla caroliniana*, in general confirmed these conclusions, but found that the response was most marked in plants which were very young at the beginning of the experiment. The response varied with the age of the plants, as to whether it was most marked on the floral or foliar tissues. Voss (46) and Griebel (18) found that extracts of tonsils, which had previously been found to have an inhibitory effect on the growth of tadpoles, had similar effects on the tissues of *Lupinus* sp. and of *Laminaria*. Griebel (18) confirmed the observations of Scheer (41) that the effect of extracts of the thymus or thyroid gland was conditioned to a considerable extent by the hydrogen-ion concentration of the medium. The experiments of Kustner (28), on the other hand, indicate that the effect of hormones may be increased by exposure to red light, not only in the Aschloim-Zondok test, but also on the germination of barley seeds treated with the urine of pregnancy.

An important contribution to the study of the action on plants of the sex hormones of animals was made by Schoeller and Goebel (42), who found that follicular hormone extracts increased the production of 'cobs' by maize plants, by increasing the number of pistillate heads, apparently at the expense of the staminate. These authors suggest that the hormone had a 'feminizing' effect on the plant. Madaus (31) was able to induce a similar effect on maize plants by using poultry hen manure as a fertilizer. All three authors have found that low concentrations of oestrogenic extracts have a stimulatory effect on the flowering of hyacinths and other plants, while high concentrations appear to increase the development of the leaf at the expense of the floral parts. Madaus has also noted a marked increase in the root-pressure of decapitated *Helianthus* plants, the roots of which were treated with adrenalin.

A very complete series of experiments was carried out by Wasicky, Brandner, and Hauke (48), who used all the readily available hormones, with hyacinths, beans, lupins, cabbages, and with the fungi *Aspergillus* and *Phycomyces*. They also repeated the experiments of Niethammer with the dormant shoots of *Forsythia*, *Prunus*, *Syringa*, *Cheiranthus*, *Crataegus*, and *Salvia*. In the main the results of these authors seem to reconcile a number of the apparently contradictory results of other workers.

The action of the hormones seems to vary to some extent with the plant and also with the concentration of the hormone itself. In high concentrations the effect of the extract may be more marked on organs and tissues other than those most affected by the same hormone in low con-

centrations. These authors found further that cod liver oil had a stimulatory effect on plant growth. They used this material because of its high content of Vitamin A. This year, however, Harden and Störmer (21) have repeated many of the experiments which have been outlined above and have found that their results have been almost entirely negative.

It is obvious from this survey that no definite conclusions on the action of animal hormones in plants can be drawn from the evidence presented in these papers and that the problem is very complicated. In the work detailed in the present paper many of the previous experiments have been repeated and a new technique adopted in an endeavour to throw fresh light on the action, if any, of these hormones on plants.

The experimental methods.

The previous work which has been summarized above was carried out, in the main, with plants which were grown in culture solutions containing the substances under investigation. In our experiments we adopted the same technique in many instances, and added to the water or culture solution in which the rooted plants were growing a suitable amount of the hormone-extract under examination. The obvious objection to this method, is that there is no certainty that the hormone in the solution enters the tissues of the plant, and if, as seems probable, some of the hormones may have a fairly large molecule, then the chance of it entering the root hairs is probably slight. It was felt that a more certain method of ensuring that the solutions entered at least into the water-stream within the plant body, if not into the actual tissues, was that used by one of the authors in earlier experiments. This consisted essentially of cutting a petiole and using the negative pressure in the water-stream to pull solutions into the plant through the petiolar stump. It has been found that this is a convenient method for introducing stains and other substances into the xylem, although it is not suggested that all substances which enter the xylem in this way are able to spread into the parenchymatous ground tissue of the plant.

In addition, a large number of experiments were set up in which the hormone substances were added to the culture solutions in which the plants were grown. For this purpose various plants were used—hyacinths, tomatoes, beans, and cabbages among others.

Experiments with oestrous hormone.

Extracts of crystalline oestrous hormone (Prolan A) from the urine of pregnant mares (glandubolin, Richter) were used in this group of experiments. A group of young tomato plants having 7–8 leaves were selected for the preliminary investigation. A leaf of each of the experimental plants was removed and the severed end of the petiole was introduced into a small test-tube of 5.5 c.c. capacity, containing either 5 c.c. of a

2 per cent. solution of glandubolin (= 100 mouse units of oestrin) or 5 c.c. of distilled water as controls. The pH of the glandubolin solution was found to be 7.24. The experiment was set up on the 23rd October. During the next few days additional glandubolin solution or water was added, as required, until by the 30th October, two treated plants had absorbed 16.2 c.c., another 18.2 c.c., and the fourth 13.2 c.c. of glandubolin solution, corresponding respectively to 327, 367, and 267 mouse units of hormone. For the first few days the uptake of the solution by the plants was fairly rapid, but it became slower as the experiment progressed. There was some evidence that the uptake of the solution was at a slightly higher rate than that of the distilled water.

The first appearance of any reaction of the plants to the treatment was manifested on the 27th October. On that date chlorosis round the veins of the leaves above the petiole was noticed. On the 1st November the top of the same leaflets showed signs of dying. The effect of the treatment was much more marked on the leaves directly above the treated petiole and on the half leaves on the same side. The leaves on the opposite side of the stem were apparently unaffected, and the leaflets in an intermediate position showed symptoms more or less severe, depending on their position with reference to the cut petiole. It was clear that the solution had moved up the stem and had entered one side of the intermediate leaves, had passed along that side to the leaflets and then had reached the other side of the leaf by way of the leaf tip. On the 6th November it was noticed that the chlorosis had largely disappeared and normal green tissues had developed right to the edge of the dead areas at the tips of the laminae.

The same plants were left under observation until the 13th November, when a second series of experiments was set up. The same method of introduction of the solutions was employed, but the leaf next above the first was removed and the cut end of the petiolar stump inserted as before into the solution. Plants 1 and 2 were given 4.5 c.c. of a solution of 2 per cent. glandubolin, this quantity corresponding to 83 mouse units of hormone, Plants 3 and 4 were given 5 c.c. each of a 10 per cent. solution of glandubolin—corresponding to 500 mouse units of hormone. Thereafter additional doses of 2 per cent. glandubolin (equivalent to 20 m. u. per c.c.) were given to all the plants, until by the 19th November the total amounts absorbed by the plants were respectively, No. 1, 373 mouse units, No. 2, 323 mouse units, No. 3, 770 mouse units, No. 4, 730 mouse units. The amounts of distilled water absorbed by the control plants was again found to be much smaller (about 50 per cent.) than those of the solutions.

The first appearance of the effect of glandubolin in this experiment was the 'burning' of the leaf-tips and the development of round, dead areas on the laminae. No yellowing, similar to the chlorosis above described,

was noted. All the treated plants, either with the large or small amounts of hormone-substance, show similar effects on the leaves in direct vascular connexion with the treated leaf. It is important to notice in these and in the other experiments dealing with the absorption of solutions into the xylem vessels, through a cut petiole, that proximity in space does not necessarily imply direct and effective vascular connexion between pairs of leaves (see 737).

It later became evident that the stronger doses had had a greater effect on the tissues than had the weaker, and the distortion and burning of the affected leaves in the plants with most hormone became very marked. A faint chlorosis was also noticeable, though not so pronounced as after the earlier treatments, but necrotic areas appeared on some of the laminae. Thereafter the dead areas became sharply delineated and the chlorotic condition disappeared. The areas immediately behind the burnt cells of the leaf-tips also made some growth, as was noted in the earlier experiment. Neither in the first nor the second experiment were the leaves below the treated petiole affected. The treated plants appear to recommence active growth at this stage and they were set aside in the glasshouse for observation. The details of the measurements made during the experiment are set out in Table I.

TABLE I.

*Experiments on the Absorption of Glandubolin through Petiolar Stumps.
(Tomato Plants.)*

Treatment of plant.	Length of stem.			
	23rd Oct.	12th Nov.	18th Nov.	10th Dec.
	cm.	cm.	cm.	cm.
Glandubolin	14.5	68.0	82.0	153.0
Control	17.0	70.0	101.0	138.0

These figures indicate that the damage to the tissues by the large doses of glandubolin does, as would be expected, reduce the growth-rate of the plant, but that this retardation is temporary, and after the glandubolin has been immobilized, probably in the leaf-tips, the plant grows at least as fast as do the controls. There were not sufficient plants to establish whether or not there was a significant increase in the growth of the treated plants, after the initial effect of the hormone had been overcome.

In order to ascertain if the hormone or products of its toxic action on the plants were still present in the tomato plants after treatment, some leaflets of those plants which had received the large doses of glandubolin were crushed in water and the macerated material was rubbed on to the leaves of normal tomato plants. The leaf hairs and portions of the epidermis of these plants were broken by this process, and the death of areas of the leaves indicated that the toxic effect of the glandubolin was

still present. Similar experiments with crushed healthy leaves and with leaves which had been burnt by being touched with a hot iron, gave no evidence of any toxic effect on the leaves of other tomato plants.

Similar experiments, in which the macerated material or a glandubolin solution 1/100 was rubbed on tobacco leaves gave no indication that the glandubolin had any effect on the tissues of this plant.

The effect of glandubolin on tomato plants in water culture.

Some young tomato plants of about 11 cm. high were put into flasks containing 30 c.c. of a glandubolin solution which contained 330 mouse units of hormone. In one plant the main root was removed. This plant first showed evidence of the toxicity of the solution, but after ten days all the plants showed signs of wilting and were obviously seriously affected by the treatment. An attempt was made to induce recovery by keeping the plants in Knop's solution, but the tissues of the roots were immediately attacked by bacteria and the experiment had to be abandoned. The evidence indicated that the absorption of relatively large doses of glandubolin through the roots of tomato plants is possible and that glandubolin is as toxic to tomatoes when absorbed through the roots as when it is administered through a petiole by the method described above.

Experiments with adrenalin.

The first series of experiments were made with adrenalin hydrochloride (Richter) in 1/1,000 solution. The pH of the solution, was found electrometrically to be about 6.8. The formula for adrenalin is $(OH)_2$, C_6H_3 , $CHOH$, CH_2 , NH , CH_3 , but in addition to the adrenalin itself the solution contained 0.9 per cent. of NaCl and 2.5 per cent. chlorotone and traces of hydrochloric acid.

The object of the first experiment was to ascertain if adrenalin had an effect on the sap pressure of plants. Madaus (31) has suggested that the root pressure of decapitated plants was much increased by the addition of traces of adrenalin to the water culture in which the roots were immersed.

Four potted tomato plants with nine leaves each were chosen for the preliminary experiment. A leaf of each was removed and the end of the petiolar stump was inserted into a test-tube. In these, as in the earlier cases, the petiole was cut under water and precautions were taken to ensure that the xylem vessels of the petiole stump would not be filled with air bubbles. With the control plants, distilled water was substituted for the adrenalin solution. After 6 c.c. of a 1/100,000 solution of adrenalin had been absorbed by each of the test plants, all four plants were put under bell jars standing on trays filled with water. The atmosphere in the bell jar soon became saturated and hydathode activity of the tomato plants was observed. It was expected that had the root-action of these plants been increased by the administration of adrenalin the hydathodes of the treated

plants would have been more active than those of the control plants and would have functioned earlier. They did not, in fact, do so, and there was no evidence that the adrenalin had any effect on the 'root-pressure'.

Observations on the plants after treatment made it clear that the adrenalin had no toxic effect on the tissues. Even after as much as 27.5 c.c. of a 1/10,000 solution of adrenalin hydrochloride had been absorbed by the treated plants, there was no evidence of any toxic action, such as was observed after absorption of glandubolin. There was, however, some evidence to suggest that the adrenalin solution was more rapidly absorbed at the cut petiole than was distilled water. It was noticed, also, that the adrenalin treated plants flowered rather more freely than did the controls, but the conditions obtaining in the glasshouses at the time of the experiments were not very favourable and no conclusion is drawn on that account. The adrenalin treated plants, certainly did not grow any faster than the controls, nor was their size affected in any way by the treatment.

The experiments on the effect of adrenalin on the 'root-pressure' was repeated with small tomato plants having seven leaves each. These were put into small flasks surrounded with black paper to exclude light from the roots. In each flask was 100 c.c. of Knop's solution alkalized with KOH to pH 8.0. To some of the flasks was added adrenalin hydrochloride to make a 1/1,000 solution, while the others were kept as controls. When these plants were put under a bell jar, as was the previous group, no difference in reaction between the adrenalin-treated and the control plants was noted. It was noted, when the plants had been in the solution for 24 hours, that the solution had developed a red colour which, however, was lost again after another two days. The addition of more adrenalin to the Knop's solution resulted in a repetition of the colouring, followed by decoloration. This coloration was not due to tomato plants alone, since similar results were obtained when cabbage and cucumber plants were substituted for them.

Neither these cabbage plants, nor detached leaves and shoots of tomato kept in adrenalin-hydrochloride solution (1/1,000) gave any evidence that the adrenalin had the effect of increasing the movement of water in the tissues. No differences of any kind could be detected between the treated and the control material, nor was there any evidence that the adrenalin was toxic to the roots or other tissues. The plants produced well-developed root-hairs in this concentration of adrenalin.

In another experiment the main root-tips of a tomato plant with ten leaves were cut off and the plant placed in a flask with 30.0 c.c. of Knop's solution with adrenalin hydrochloride added to give a concentration of 1/10,000. The plant wilted slightly and then absorbed all the liquid in the flask. Thereafter it was kept in Knop's solution, where it grew quite normally and showed no evidence of toxic effects of the earlier treatment.

A solution of adrenalin hydrochloride from the British Drug Houses was also used in some of the experiments. It was found to have a pH of 5.33 at a concentration of 1/1,000 in water. Young tomato plants were kept with their roots in flasks in 30 c.c. tap water for a few days and then to the water was added 0.1 c.c. or 1.0 c.c. to a 0.1 per cent. solution of adrenalin hydrochloride, corresponding to a concentration of adrenalin hydrochloride of 1 in 3,000 or 1 in 300. In these high concentrations the red colour was very markedly produced (the colour at the lower concentrations was safrano pink (Pl. II, 7-R-O, f) and at the higher concentrations grenadine (Pl. II, 7-R-O, c) of Ridgeway's Colour Standards). The colour disappeared again after a few days, and while at the higher concentration of adrenalin hydrochloride the roots appeared to be somewhat shrivelled, the new roots which developed later produced normal and plentiful root-hairs. No evidence was obtained which suggested that the adrenalin hydrochloride had any toxic effect on the plants.

Experiment with the extract of the anterior lobe of the hypophysis.

This extract is prepared by Richter under the name of 'glanduantin'. Four medium sized tomato plants were selected for the experiment. Two were treated with glanduantin and two were kept as controls. There were left on each plant three flower trusses, the lowest with three flowers, the second with four buds, and the uppermost with six very small buds developing. Through the severed petioles of a leaf on each of two plants 170 rat units of glanduantin was administered as an aqueous solution, while the controls received only distilled water. There was no sign of a toxic action of this substance, even at the very high concentrations in which it was used. Measurements were made of the plants and these are detailed in Table II.

TABLE II.

Plant.	Height.	No. of leaves.	Weight of fruits.	Weight of stem and leaves.	Weight of roots.
	cm.		gm.	gm.	gm.
No. 10 Treated	170.5	53	116	282	44
No. 12 „	155.0	57	135	264	35
No. 11 Control	169.0	52	102	255	72
No. 13 „	167.0	58	0*	267	61

* Ovules not fertilized.

It would appear that this extract, while it had no effect on the foliage of the plants, may have, as suggested by Wasicky, Brandner, and Hauke (48), who worked with French beans, an effect on the differentiation of tissue, and the very low weight of the roots of the treated plants is to be noted.

Before the plants were weighed another 100 rat units of glanduantin

was administered to each by cut petioles, so that in all they had each 270 rat units of glanduantin, which induced no toxic effects on the foliage.

Experiments with testis extract (orchitic, Richter).

Experiments were set up to discover whether testis extract would show the same toxic effect as did the extracts of follicular hormone. Medium sized tomato plants were selected and doses of 1 or 2 c.c. of testis extract in 4 or 3 c.c. of water respectively were introduced through cut petioles. The subsequent growth of the plants was apparently neither accelerated nor retarded by the treatment.

The experiment was repeated with very young rooted tomato plants, grown in water, which were equally unaffected by solution of testis material. The Richter extract was so prepared that 1 c.c. of extract was equivalent to 15 gm. fresh testis tissue.

Experiments with extract of the suprarenal cortex.

In an attempt to discover the effect of the hormone on the suprarenal cortex on the sap-pressure of tomato plants the experiments outlined above for adrenalin were repeated with cortex extract (Richter's 'cortigen'). This was used in a solution of 1/1,000 with water cultures, and 1/10 or more concentrated in experiments involving absorption through cut petioles. Cortigen Richter is so prepared that 1 c.c. is equivalent to 16 gm. of fresh suprarenal cortex. The pH of a 1/100 solution in distilled water was 7.3. In none of the experiments did cortex extract appear to have the slightest effect of any kind on the treated plants.

Experiments were set up to see whether very high concentrations of cortex extract had a toxic effect on tomato plants when introduced through cut petioles. Plants of various sizes were selected and were treated with cortigen solution of concentration of 1/10 or 2/5 in distilled water. As much as 10 c.c. of the solution in the higher concentration, i.e. 4 c.c. of cortigen have been absorbed without any apparent differences being manifested. So far, therefore, as tomato plants are concerned cortigen has no demonstrable toxic effects.

Similar results were obtained when broad beans (*Vicia faba*) were grown in Knop's solution containing cortigen in concentration of 1 in 125 and 1 in 10. At both concentrations the leaves appeared to grow as normally as did the controls in Knop's solution.

Experiments on the effects of gland extracts on the growth of Hyacinths.

A group of hyacinth bulbs of the variety 'L'Innocence' which had been prepared for forcing, were kept on damp moss, covered with pebbles in a dark cellar until the roots had begun to grow. They were put in the cellar on the 1st November, 1934, and had all developed roots

by the 13th November. On that date they were put on wide-necked bottles of 300 c.c. capacity. These were filled with tap water, covered with black paper and put in a warm greenhouse under a black shade. On the 19th November, the leaves having grown to some half-inch or so, the shade was removed and the bulbs were divided into five groups, all the groups being, so far as possible, identical as regards the development of roots, leaves, and the general appearance of the bulbs. Some days before (19th November) while the plants were still in the dark, solutions of glandular extracts were added to the bulbs as indicated in Table III.

TABLE III.

The Amount of Glandular Extract added to Cultures of Hyacinths.

Serial number.	No. of bulbs in series.	Extract.	Concentration of extract.	Amount per flask. c.c.	pH.
I. a.	3	Glandubolin (follicular hormone)	100 M. U.	2.0	7.5-8
b.	3	" " "	" "	5.0	—
II. a.	6	Orchitic extract	16 gm./1 c.c.	1.0	7.5-8
III. a.	3	Thyroid gland	16 gm./1 c.c.	1.0	7.5-8
b.	3	" "	" "	2.0	—
IV. a.	2	Adrenalin	1/1000 aq.	0.5	—
b.	1	" "	" "	1.0	6.8
c.	3	" "	" "	0.3	—
V.	6	Control: (1) Peptone	1/1000	—	8.0
		NaCl	8/10000	—	—
		(2) Special meat extract	" "	3.0	7-7.5

These solutions were made up on the 9th November and were changed on the 7th, 11th, and 17th December. On the 22nd December one-third of the usual concentration was used, and on the 27th December one-half. The usual treatment was repeated on the 3rd January 1935. It was found that the peptone-salt solution used as control was unsatisfactory as bacterial and fungal growth was extensive, even after a week, and a special meat extract—prepared by Richter and hormone-free was substituted.

A few hours after the first administration of the adrenalin, the solution was coloured pink, this colour being darkest in the most concentrated solution. Gradually this colour disappeared, as happened also with tomato, and was not observed in the subsequent treatments with adrenalin. On the 28th November, when the bulbs were placed on a glasshouse bench in the light, the leaves of all were approximately equal in development with those of the group receiving thyroid extract, perhaps slightly in advance. The flowering times of the bulbs is indicated in the following table.

It was noticed that the control and the treated plants developed, at first, at the same rate. After the appearance of the flower buds, however, the treated plants were found to be slightly in advance of the controls (see

Fig. 1). When fully grown, however, there was no marked difference in the size of the plants, though the leaves of the thyroid and adrenalin groups appeared to be rather less well developed than those of the 'orchitic' and 'glandubolin' groups, the difference between treatments manifesting itself rather in the rate of development. It is interesting to notice in this connexion that the whole inflorescence was completely wilted nineteen days after the opening of the first flower in all the plants, regardless of the stage in the experiment at which they opened. There was no evidence that differences in concentration of the hormone extracts had any effect on the growth of the experimental plants (see Table V and Graph).

TABLE IV.

Time of the Appearance of Flower Buds of the Hyacinth Bulbs.

Treatment.	Date.			
	7th Dec.	10th Dec.	12th Dec.	18th Dec.
Glandubolin (a)	1	2	2	3
(b)	0	1	2	2
Orchitin	3	4	5	5
Thyroid extract (a)	2	2	2	2
(b)	2	2	3	3
Adrenalin (a)	0	0	0	1
(b)	0	1	1	1
(c)	0	2	3	3
Controls	2	2	2	3

TABLE V.

Effect of Hormone Extracts on Hyacinths.

(a) Height of plants in cm. 30/1/35.

Controls.	Adrenalin.	Thyroid.	Orchitic.	Glandubolin.
31.5	30.5	32.5	29.0	33.0
29.5	30.0	29.0	26.5	25.0
28.0	29.5	28.5	27.5	34.0
19.5	27.0	29.0	33.5	24.5
24.0	27.0	19.0	32.0	27.0
Average 26.5	29.2	27.6	29.7	28.7

(b) Weight of leaves. 30/1/35.

27.0	35.0	34.0	27.0	38.5
26.0	30.0	32.0	18.0	22.5
23.5	37.0	36.5	20.0	30.0
16.5	33.5	38.5	47.0	16.0
23.0	21.0	33.5	29.5	23.0
Average 23.2	31.3	34.9	28.4	26.0

To test the effect, if any, of the solutions on detached leaves of hyacinth, leaves from the control plants were cut off and put in the following solution—(1) 19 c.c. of distilled water and 1 c.c. glandubolin (= 1,000

mouse units), (2) 20 c.c. of a 1/50 solution of adrenalin hydrochloride (0.1 per cent.), (3) 20 c.c. of a 1/10 solution of the control extract (see below, and (4) 20 c.c. distilled water. The control extract damaged the

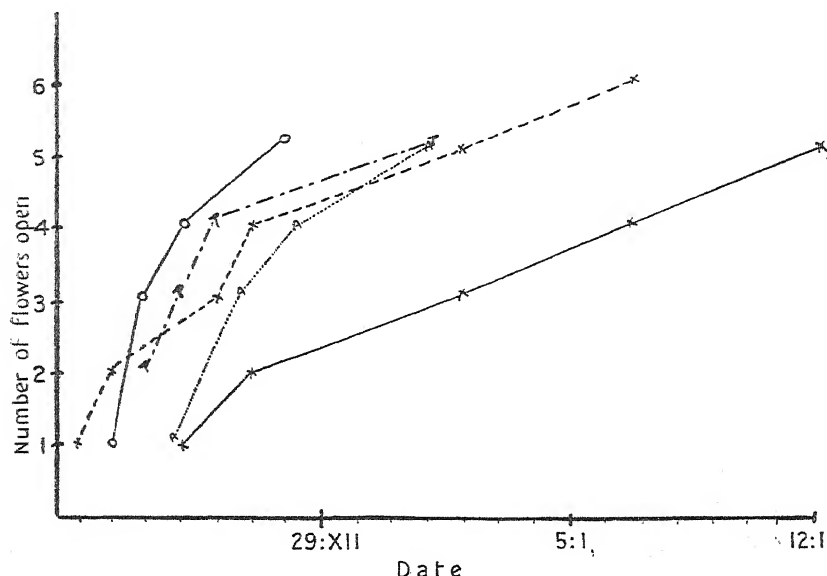


Chart illustrating time of flowering of the treated hyacinths. x—x Control. x---x Glandubolin. O—O Orchitic extract. A---A Adrenalin. T---T Thyroid extract.

cut surfaces of the leaves and the tissues around them, the adrenalin solution turned reddish and then discoloured. None, except the 'control extract' solution appeared to affect the leaves.

Experiments with 'Control Extract'.

It was thought desirable to set up a series of control experiments with an extract of muscle tissues which nearly resembled the hormone extracts in chemical composition. By the courtesy of Dr. Gerö of Richter Ltd., such a substance was prepared, and put up for convenience in 1 c.c. ampoules each ampoule containing the equivalent of 5 gm. of fresh muscle substance.

This material was used in methods similar to those employed with the hormone extracts. When concentrated doses of the extract were absorbed by cut petioles (e.g. 5 c.c. extract-0 c.c. H₂O, 2 c.c. extract-3 c.c. H₂O, and 1 c.c. extract-4 c.c. H₂O) the petiolar stump was plasmolysed and soon collapsed. The stem below and above the treated petiole also showed signs of disturbance round the associated vascular bundles, and deep grooves were formed for distances of as much as 80 cm. above the treated

petioles. These grooves extended a short distance into the petioles of other leaves in direct vascular connexion, but in no case examined did the leaflets appear to be affected by the treatment. When more dilute concentrations were used, the amount of damage was proportionately less, and at concentration as low as 1 c.c. in 500 c.c. of solution = 1 per cent. of original muscle tissues, no damage to, or stimulation of, the plants was recognizable. In all cases where damage was noted, the extent was limited in that the cells of the medulla and ground-tissue around the damaged area were stimulated into activity, and a layer of phellogen cells formed. Plants which, after the formation of the 'grooves' in the stem, were allowed to continue growth, while they did not regenerate the dead tissues, developed perfectly normally in respect of the rest of the plant.

In a series of plants, capsules having fine capillary ends, and filled with the undiluted extract, were inserted into the vascular tissues, and in spite of the fact that only very small quantities of the material was absorbed (e.g. 0.2-0.3 c.c.), the damage to the bundles was similar to, if not as extensive as, that discussed above.

Young rooted plants were put into solution of the extract of concentrations 1/10 and 1/100. In the weaker solution no effect was evident after some days' treatment, while in the more concentrated solution, the roots were shrivelled within twenty-four hours, and the plants ultimately died. In these, as in other experiments, fungi and bacteria tended to grow in the solutions, and prevented the extension of the observation over a period of more than a few days.

DISCUSSION.

In a discussion of the experimental results outlined above, the limitation of investigation of the response of plants to animal hormones must be considered. The extent, and even the nature of the response of animals themselves to the artificial administrations of these hormones, is not always regular, and varies with a number of internal and external factors, many of which are as yet not comprehended.

Moreover hormones, with a few exceptions, are not synthetically prepared and obtainable in a chemically pure form, so that the composition of extract of glandular material must vary very much, depending on the method of extraction. This may well account for the many apparently contradictory results which have been obtained by workers, using, nominally, the same materials. At the same time, the relatively simpler organization of plants, may complicate the investigation and interpretation of the effects of substances which have only very markedly specific effects on certain of the highly differentiated animal tissues.

How far plants themselves secrete substances similar in character to the animal hormones has not been fully established. Various workers

from the time of Dohrr, Faure, Poll, and Bloteuogel to Blütenandt (4, 5) have found, however, that oestrogenic substances ('thelykinins') are present in a great many plants. These induce oestrus in mice (as shown by the Allen-Doisy test) in the same way as do oestrogenic substances of animal origin. Kogl and Haagen-Smits' 'auxin' (1931) is a further indication of possible parallelisms between the two groups.

It is reasonable to suppose, therefore, that some at least of the animal hormones might have a specific effect on the protoplasm of plants. It is hardly to be expected that the effects need be generalized, or that they would be manifested under every condition and after administration, for example, through the root system. It is hardly to be expected that the effect of adrenalin, for instance, need be the same when it is absorbed through the digestive tract as when injected intravenously into an animal, or that oestrogenic substances would act equally well if smeared on the epidermis of the experimental animal.

With these considerations in mind, we have to examine the data outlined above to discover if animal hormones affect plants at all, and if they do, does the effect show itself in the precocious development of tissues, the marked increase in size, or in an alternation in the proportion between the reproductive and vegetative parts.

The rather drastic method adopted of introducing relatively enormous quantities of hormone substance into a plant by means of a petiolar stump, resulted in considerable damage to the plant when oestrogenic substance (prolan A) was so administered. Similar concentrations of other hormone extracts, such as testis hypophysis, suprarenal and suprarenal cortex extracts, did not induce similar damage to the tissues. The preparations were clearly of different toxicity with reference to the same test plants.

It was clear from the experiments with oestrogenic substance that this extract moved freely with the water stream, and was able, moreover, to leave the xylem elements and to enter the parenchymatous ground-tissues. It is to be noted, on the other hand, that the toxic effects of concentrated solutions of hormone-free tissue extract were, in our experiments, limited in every case to the tissues round the xylem of the stem, and for some reason not yet clear, were not manifest in the tissues of the leaves of the treated tomato plants. It is further of interest to note that the toxicity of the prolan A group oestrogenic substance, was observed when the substance was introduced through the petiolar stump, or through the roots in water-culture.

Not all our results, however, gave indication that the effects described by other investigators were to be found in the plants used in our experiments.

The effects of the hormone extracts on growth.

The effect on growth of the oestrogenic substance is indicated by the fact that the tomato plants, after recovering from the tissue damage caused by its absorption, grew actively, and developed a larger root system than did the controls, while the size of the aerial parts was as great as that of the controls, even in spite of the check they had received. There was evidence, also, that the very large doses of thyroid extract given to the hyacinths induced an increase in size. On the other hand, extracts of testis and of the anterior lobe of the hypophysis, which in animals are supposed to induce increase in size, failed to produce a similar effect on the treated plants, while in the case of the hypophysis extract there were indications that the root systems had been decreased. The absence of effect on growth of the extracts of the suprarenal and suprarenal cortex in our experiments, while in accord with the findings of others in experiments with animals, are not in accord with those of many of the workers on plants, whose papers are noted in the introduction. As regards the extracts of the suprarenal cortex, some preliminary experiments on germination with peas and wheat seemed to indicate an inhibitory effect induced by this substance.

The effect of adrenalin on the root pressure.

Although adrenalin has a specific effect on the blood-pressure of animals, and has been said to increase the root-pressure of plants, our experiments with the substance have yielded negative results. Nor was there any evidence, from experiments with slices of the tissue of stems of tomato plants, that this substance had any effect on the respiratory mechanism when administered in considerable concentration (1 in 300). It was repeatedly noticed, however, that the enzymes in plant tissues had an interesting effect on this substance. When rooted plants, the petioles of detached leaves, or extracts of plant tissues were placed in adrenalin solutions, the solutions after a few hours developed a reddish coloration, which after a longer or shorter period disappeared. The reaction was very marked with extracts of potato and similar tissues which are known to have a high oxidase content, and it is suggested that the oxidase system acts on the adrenalin, and produces from it a coloured, and later a colourless substance similar to those produced by the oxidase systems on the catechol substance in the potato, for example.

The effect of the hormone extracts on the floral development.

It is recognized that some hormones have a very marked effect on the reproductive organs of animals. In our experiments with tomato plants, however, there was no indication that any of the hormones used, including

testis and ovary extracts, had any effect whatever on the flowering. There are indications, nevertheless, that some of the hormones used may have induced increased flower development in hyacinths. With hyacinths, on the other hand, the thyroid extract gave no evidence of any effect in either direction. It may be significant, being contrary to the findings of others, that it was noted that the tomato plants which had been treated with extracts of the suprarenal and the suprarenal cortex appeared to flower more freely than did some of the controls grown under similar conditions, the conditions in the glass-houses in winter not tending to the production of flower-trusses.

CONCLUSIONS.

The obvious objection to the administration of hormone extracts to plants, is that with the hormone itself is being given material which might have a definite nutritive value, making the interpretation of results difficult. To overcome this difficulty we have used the muscle-extract which was described above. This was prepared as a hormone-free substitute for the glandular extracts with which we worked. The experiments with this material did not give any indication that this substance actually increased the growth or had, in fact, any marked effect on the plants, except when used in concentrated solution, when it was actually toxic. It is to be noted, further, that the reactions observed are not necessarily specific to hormones but, this is not unexpected, since many chemical substances are known to induce in animals themselves reactions comparable with those of hormones.

From our experiments, therefore, it seems reasonable to conclude that while many hormone extracts apparently have no effect on the growth and tissue differentiation of plants, others may have effects which may be stimulatory or toxic.

SUMMARY.

The present position of our knowledge of the effect on plants of animal hormone extracts is examined. Experiments are described in which different glandular extracts were administered to plants, either by the roots or by the petiolar stumps, and the results obtained and discussed. Glandubolin-Richter, a preparation of oestrogenic hormone, was found to be toxic to tomato plants when administered through a cut petiole or through the roots, but even concentrated extracts of the tissue of testis, ovary, pituitary, suprarenal, thyroid, and thymus appeared to have no marked effect, and certainly no toxic effect on the plants. The results obtained indicate that most animal glandular extracts have little or no effect on the growth and development of plants, but suggest one or two fruitful lines for further investigation.

We desire to express our gratitude to Sir John Russell and Dr. J. Henderson Smith for their kindness in offering facilities for the carrying out of this work, and to our colleagues for their help, especially to Fraulein Cunow and Dr. H. Nicol. Our thanks are due, also, to Messrs. Richter of Budapest, and to their Director in London, Dr. J. Gerö for the material furnished.

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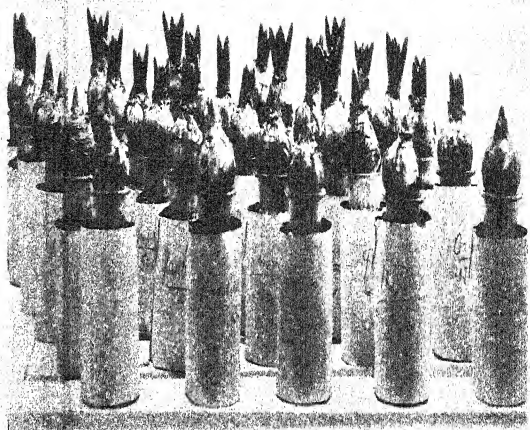
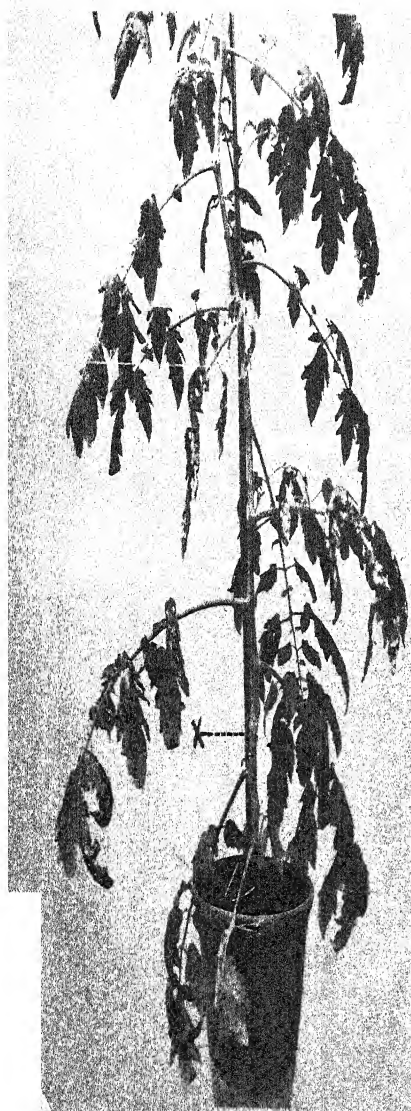
EXPLANATION OF PLATE XV.

Illustrating the paper by Mr. L. Havas and Dr. J. Caldwell on 'Some Experiments on the Effects of Animal Hormones on Plants'.

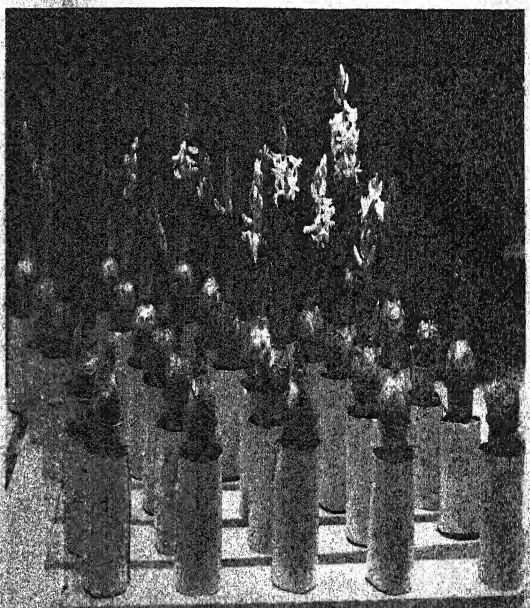
Fig. 1. Tomato plant after treatment with 'Glandubolin' (oestrogenic hormone, preparation). The hormone solution was absorbed at the petiolar stump X.

Fig. 2. Photograph taken on 15th Dec. of the treated hyacinths. The treatments were as follows: (a) Controls, (b) Glandubolin, (c) Adrenalin, (d) Thyroid extract, (e) Orchitic extract.

Fig. 3. Photograph taken on 25th Dec. of the treated hyacinths. The treatments were as follows: (a) Controls, (b) Adrenalin, (c) Thyroid extract, (d) Orchitic extract, (e) Glandubolin.



2



3

A Study by Statistical Methods of the Distribution of Species in Grassland Associations.

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With thirteen Figures in the Text.

CONTENTS.

	PAGE
I. INTRODUCTION	749
II. EXPERIMENTAL RESULTS:	
Data of Distribution Curves	752
Relationship between Quadrat Size and Percentage Frequency	759
Relationship between Percentage Absence and Density of Population	763
III. DISCUSSION	766
IV. SUMMARY	772
LITERATURE CITED	773
APPENDIX	775

I. INTRODUCTION.

IN spite of its fundamental importance the problem of the exact distribution of species in a plant association has never been subjected to critical analysis. In this country little, if any, attention has been given to the subject, such work as has been carried out has been done in the Scandinavian countries, and in Switzerland and America. Raunkiaer (23, 24) and Jaccard (12-15) in early investigations sought some numerical standard of the relative occurrence of species in an association. Their main object was to define more precisely such ecological terms as dominance and subdominance. The definitions adopted by both workers were based on a comparison between the percentage frequencies of occurrence. These frequencies were determined by noting the occurrence of each species in a number of quadrats scattered over the association. Both Raunkiaer and Jaccard found that if these percentage frequencies were divided into a number of classes, then the distribution in these classes appeared to be of the same order irrespective of the type of association. Raunkiaer, dividing the percentage frequencies into five categories, found that the distribution curve

was U or J shaped; the rarest (species with a percentage frequency of less than 20 per cent.) and the commonest (percentage frequency greater than 80 per cent.) being present in the largest numbers. Vahl (30, 31), Palmgren (21), and, more recently, Kenoyer (16) have confirmed these results. Jaccard, on the other hand, in his studies on alpine meadows showed that while a very large proportion of rare species were present there was not in all cases a large number of dominant species. Ashby (4) in a recent note has criticized these workers on the grounds that the frequency classes into which they divided the data are misleading, since it can be demonstrated mathematically on the assumption of a random distribution that these frequency classes are not of equal size.

Arrhenius (1-3), Gleason (8-10), Woollet, Dean and Coburn (34), and Hanson and Ball (11) have studied the relationship between the size of the quadrat and the average number of species found within the quadrat. These investigators showed that as the quadrat area was increased the rate of increase in the number of species found was at first high, but gradually fell off as the area became relatively large. Arrhenius (1, 3) and Gleason (9, 10) evolved empirical formulae to fit the data they obtained. The treatment of the data by Arrhenius and by Gleason is open to serious objection and will be referred to again later.

Du Rietz, Fries, Osvald, and Tengwall (6) developed, at the same time, some other relationships between species and area. They carried out a series of observations on the changes in the percentage frequency as the area of the sampling quadrat was increased. They claimed that with increasing area the number of species with an observed frequency of 90 per cent. or over tended to become 'constant'. They called the quadrat size in which the 'constant' species reached a frequency of over 90 per cent. the 'minimal area' for the association. These authors contended that associations were well-defined units with limited 'constant' species, and claimed that the number of such species could be used as a criterion of classification. The claims of these workers have been criticized by Arrhenius (3) and Pearsall (22), who have shown that by increasing the area beyond the minimal size as defined by the method of du Rietz, Fries, Osvald, and Tengwall, further species become 'constant'.

Mathematical formulations of the relationship between species and quadrat area and of the distribution of percentage frequencies have been put forward by Romell, Arrhenius, Kylin, and Wicksell. Romell (25-28) based his calculations on the premise that the positions of the various plants depended upon their suitability to their habitat, each species needing for successful establishment a definite combination of factors in the external environment. By making a number of assumptions concerning these factors, he stated that it could be demonstrated mathematically that the results of Raunkiaer, Jaccard, and Palmgren were in agreement with his

hypothesis. Kylin (17) and Wicksell (32) in two purely mathematical papers claimed that the relationship between species and area and the distribution of frequencies were in agreement with the theory of probability of occurrence. Neither author included any field data; each was content to demonstrate that the theoretical curves were of the same order as those obtained by other workers from field observations.

It must be pointed out that in a plant association any mathematical treatment of the data of distribution for each species depends for its validity on a knowledge of the nature of the distribution. *The calculations of Wicksell, of Kylin, and of Arrhenius hold good only if the individual plants of each species are distributed at random in the association.* These workers do not, however, offer definite proof of the absence of correlation between individuals. Kylin and Wicksell do not compare their theoretical curves with those derived from field data. Furthermore, Svedberg (29) found in a study of seven species, each in a separate association, that only in four cases were the individuals 'normally dispersed'.

The following investigation was therefore undertaken to determine in what manner the species in grassland associations are distributed. It was considered improbable that the distribution would be of the same type for all species, since the differences between the methods of reproduction are so great, e.g. those between a stoloniferous plant and one depending on the wind for seed dispersal. If, on the other hand, it could be determined in what manner the majority of the species were distributed, it would allow of a fuller and more satisfactory interpretation of the data collected by existing methods in the 'botanical analysis' of pasture.

A statistical study of distribution is essentially a study of a population. In such a study attention is often confined to one characteristic in the individual and the population is divided into frequency classes defined by the range of the variate chosen, e.g. ranges of height in man. In a plant association the population can also be divided into frequency classes, defined in this case by differences in the density of population. In cases where it is possible to count individual plants, the frequency classes of the variate density may be expressed as classes of nought, one, two, or three occurrences of a given species recorded in sample areas of any convenient size.

In grassland associations there are a large number of plants in which it is not possible to count individual plants since they are propagated either by stolons or rhizomes; in these other means of determining the density must be used. In such cases the *percentage area* covered by each species was estimated or, alternatively, the number of tillers was counted.

II. EXPERIMENTAL RESULTS.

Data of distribution curves.

Collection of data. Since the object of the investigation was to determine the nature of the distribution of species in relation to the analyses of small, experimental plots, the area from which the random samples were taken did not exceed 0.02 of an acre. Sampling was carried out by means of a 6 in. \times 6 in. quadrat in the majority of the investigations. This particular shape and size was chosen because such a quadrat is already widely used in England by workers interested in pastureland problems. In each set of observations on distribution 110–500 samples were taken.

Determinations of the percentage area covered by the species were made by eye. In order to facilitate this estimation, the quadrat was subdivided into a number of smaller areas by means of fine wire. For tiller counts, 6 in. \times 6 in. turves were removed to the laboratory, the plants teased apart, and the number of tillers for each species noted.¹ Where it was possible to define individual plants, no account was taken of those cases in which less than half a plant fell within the quadrat.

Discontinuous distribution curves. As has already been pointed out, when it is feasible to count individual plants the frequency classes may be expressed as nought, one, two, three, &c., occurrences of the species in the sampling quadrat. The variable being the number of occurrences, it can assume only values which are whole numbers and the distribution is discontinuous. Of these discontinuous distributions, the Poisson Series is one of the best known, and the values for frequency of occurrence in the classes are given by the series

$$e^{-m}, me^{-m}, \frac{m^2}{2!} e^{-m}, \dots, \frac{m^x}{x!} e^{-m},$$

where m is the mean frequency of occurrence in the sample. The terms in the series give the values of frequency of occurrence of the chosen species; thus e^{-m} will be the proportional number of samples containing no individuals of the species, me^{-m} the number of times one individual is present, $\frac{m^2}{2!} e^{-m}$ the number of times individuals of the species will occur twice, and so on.

If individuals of a species are distributed at random, it is to be expected that distribution curves constructed from field data would conform to the Poisson type. In Fig. 1 a number of distribution curves are plotted from data collected from alpine pastures in the neighbourhood of St. Anton-am-Arlberg (Austria). Individuals of the species in question could easily be counted, as propagation appeared to be almost entirely by seed.

¹ For these data I am indebted to Mr. William Davies of the Welsh Plant Breeding Station, who carried out this laborious investigation.

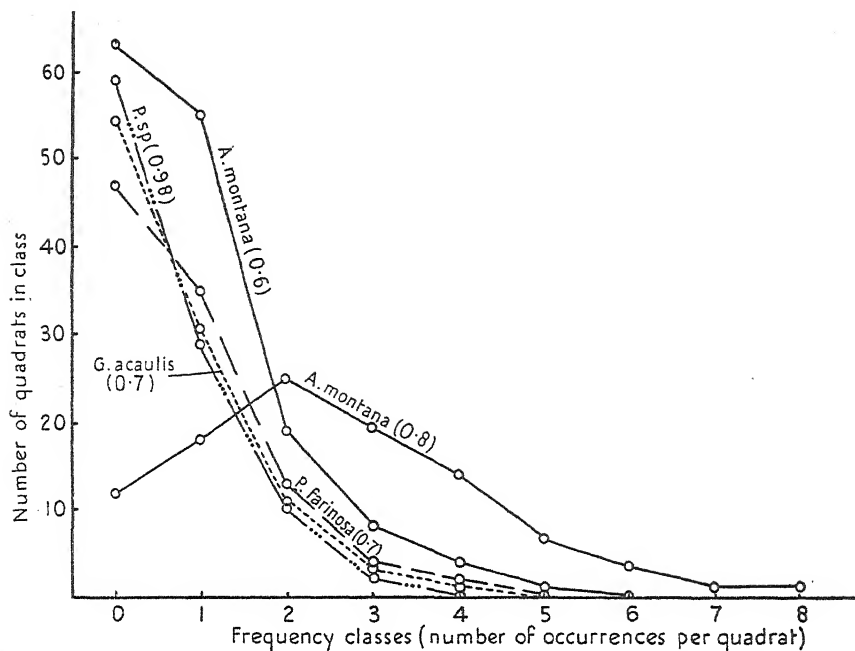


FIG. 1. Distribution curves of density for a number of alpine meadow species. The frequency classes are expressed in terms of the number of occurrences in the sampling quadrat. The figures in brackets refer to the value of P as determined by the χ^2 test for a random distribution of the Poisson type. Data collected St. Anton-am-Arlberg, October 1933.

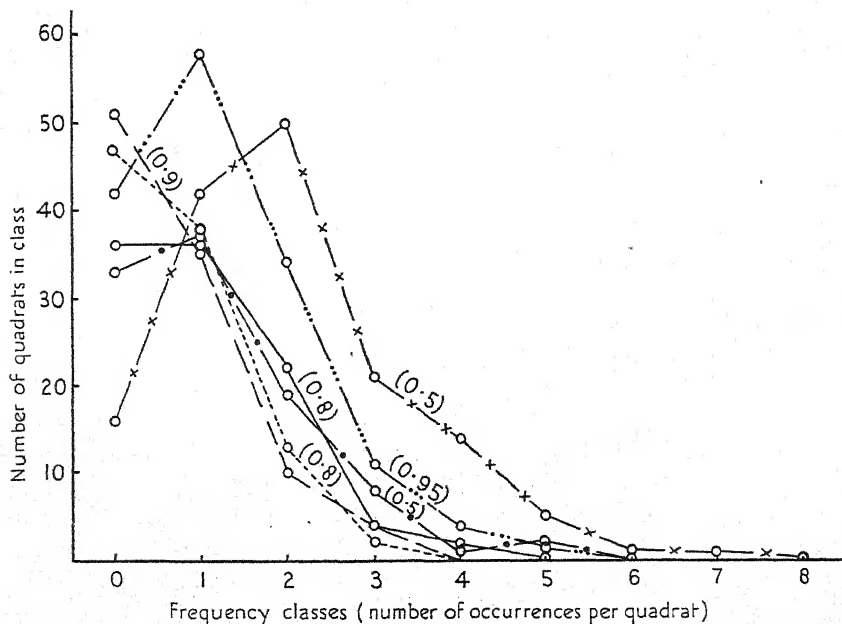


FIG. 2. Distribution curves of density for *E. maritimum* in a number of similar associations. The frequency classes are expressed in terms of the number of occurrences in the sampling quadrat. The figures in brackets refer to the value of P as determined by the χ^2 test for a random distribution of the Poisson type. Data collected St. Jacut-de-la-Mer, France, October 1934.

In order to test how closely these curves conformed to the Poisson type, the χ^2 test (see Fisher (7)) has been applied, and the value of P for each curve is shown in brackets in Fig. 1. These values of P represent the probability (stated as a decimal fraction) that a discrepancy at least as great as that found between the calculated and observed values should have arisen solely by chance. The smaller, therefore, the value of P , the less evidence is there for the observed distribution being of a Poisson type. For purposes of judgement, the limit of P chosen, beyond which the agreement is too poor to substantiate the hypothesis, is 0.05. Since P is markedly greater than 0.05 in each case, the distribution appears to be of the Poisson type and at random.

In Fig. 2 distribution curves are shown for *Eryngium maritimum* based on data collected from a number of different localities in the neighbourhood of St. Jacut-de-la-Mer (Brittany). In each case the dominant grasses in the sward were *Festuca rubra* and *Elymus arenarius*. The quadrats used in this series of observations were 45 cm. square and the area on which the observations were made was in each locality about 100 square metres. The values of P (vide Fig. 2) indicate that the distribution was at random in each of the observed areas in spite of the fact that the reproduction was not wholly by seeding.

If individuals of a species are in some way markedly correlated then it is not to be expected that the distribution should be at random. In Fig. 3 a distribution curve for *Primula auricula* is shown. In this species there is vegetative reproduction by short rhizomes and the older individuals tend to be surrounded by younger plants. It is seen that the calculated Poisson distribution does not fit that constructed from the field data, P being less than 0.05; the distribution therefore is not at random.

In Fig. 4, distribution curves are given for *Plantago media*, collected from four areas (each 35–60 square yards) in the same association. In this species reproduction is largely through seeding, but the values for P (vide Fig. 4) show that the distribution is not at random. The most marked discrepancy between the field data and the calculated figures was the paucity of the number of quadrats containing a single plant and the excess containing 0, 3, 4, and 5 plants, suggesting that the plants occurred in clumps. This was confirmed by observation in the field.

It has already been pointed out that in grassland associations it is impossible to count individual plants. In such cases, estimations of the density in the sampling quadrat must be made by other means. When the sward is not closely grazed then counting the number of tillers of each species in the sampling quadrat (6 in. \times 6 in.) is often used. Since the variance is not continuous, it is to be expected that distribution curves obtained from such data should be of the binomial type. In Fig. 5 distribution curves have been drawn from the data collected from a permanent

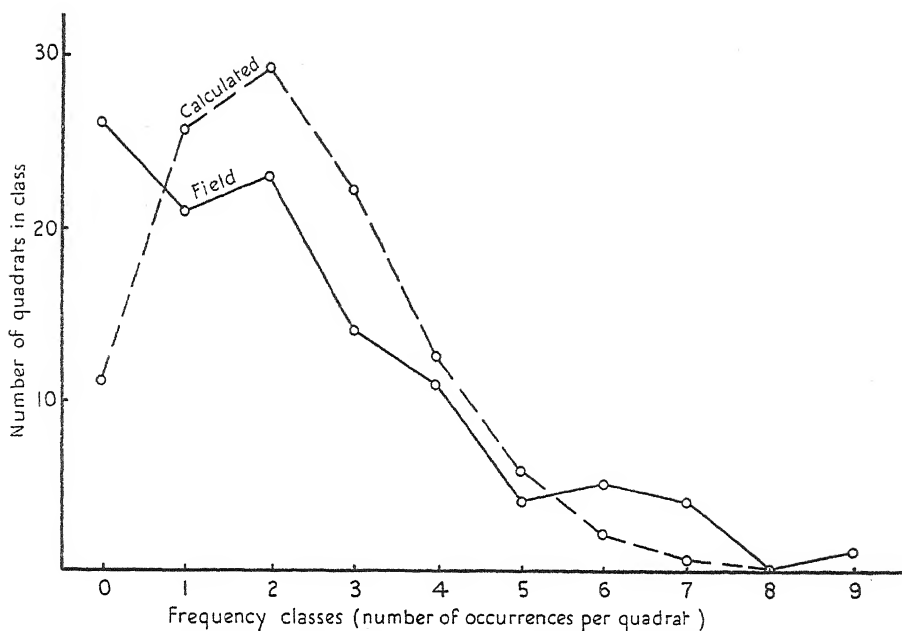


FIG. 3. Distribution curve of density (number of individuals per quadrat) for *P. auricula*. For comparison the calculated distribution curve based on a random distribution of the Poisson type is also given. The agreement is not significant. Data collected St. Anton-am-Arlberg, October 1933.

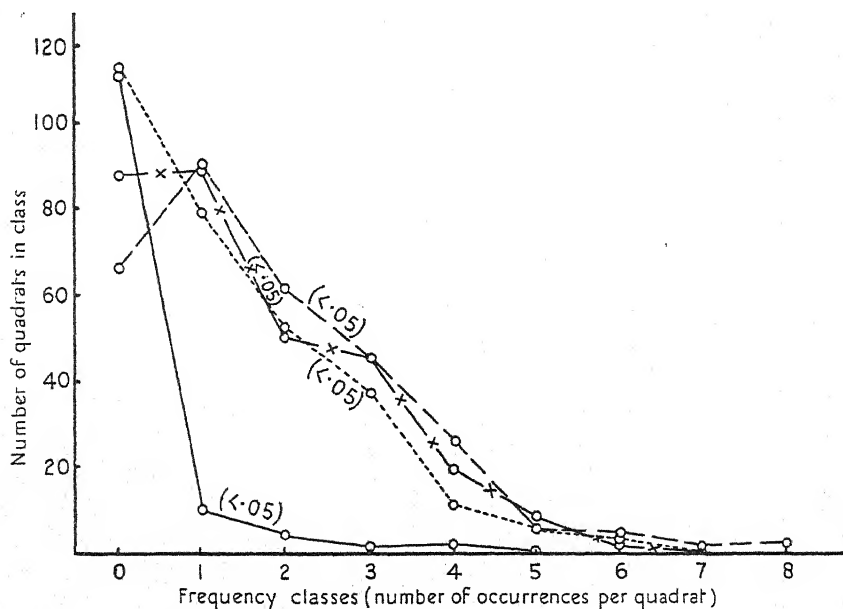


FIG. 4. Distribution curves of density for *P. media*. The frequency classes are expressed in terms of the number of occurrences in the sampling quadrat. The figures in brackets refer to the values of P as determined by the χ^2 test for a random distribution of the Poisson type. Data collected Cookham, October 1934.

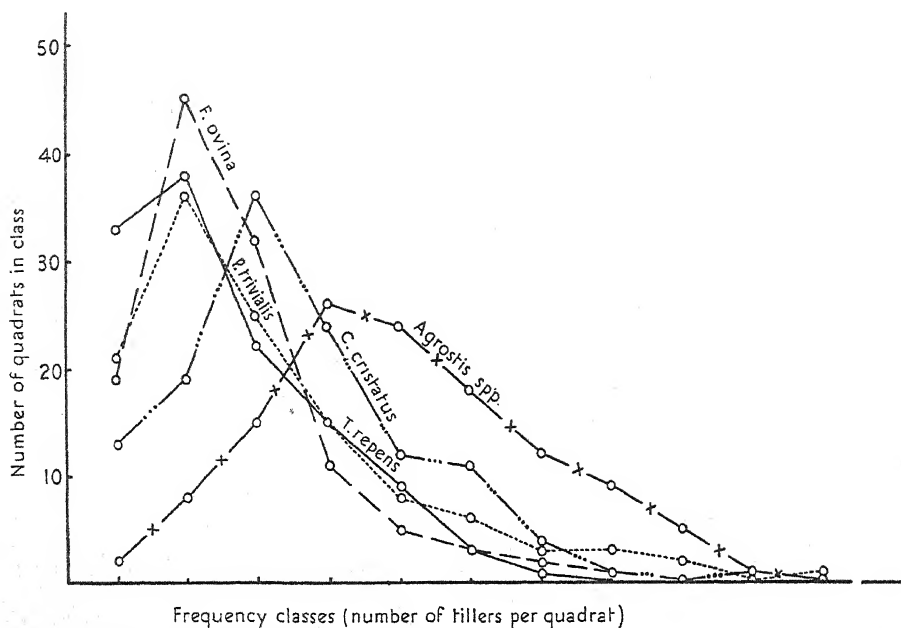


FIG. 5. Distribution curves of density for a number of species in the same association (Aberystwyth pasture). The estimate of density was obtained by counting the number of tillers in the sampling quadrat. The frequency classes for the various species are: *Agrostis* spp. and *C. cristatus* 0-19, 20-39; *F. ovina* 0-9, 10-19; *P. trivialis* 0-14, 15-29; *T. repens* 0-6, 7-13, tillers per quadrat.

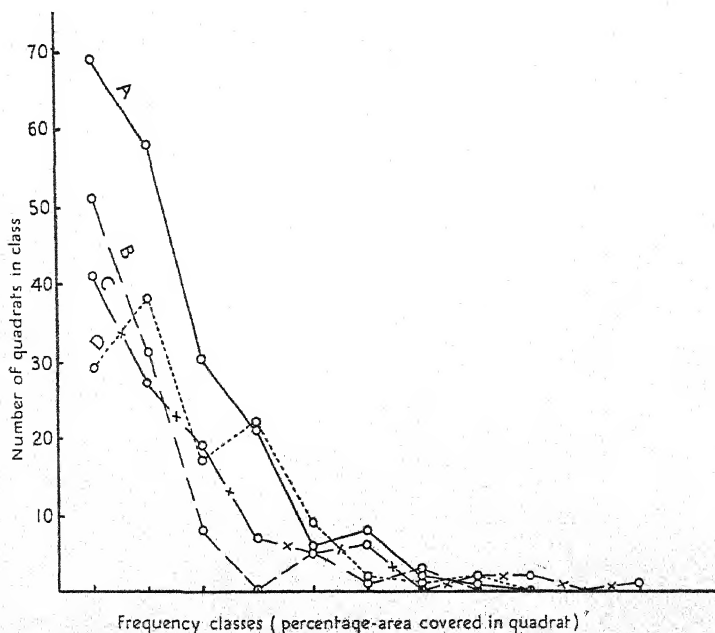


FIG. 6. Distribution curves of density for *T. repens* in four different associations. The estimate of density is based on the percentage area covered by the species in the sampling quadrat. The frequency classes for the four curves are: A, 0-5, 6-11%; B, 0-3, 4-7%; C, 0-1, 2-3%; D, 0-9, 10-19%. Data collected Jealott's Hill, May 1930-June 1932.

pasture near Aberystwyth. In this figure distribution curves are shown for the chief species in the sward. Each species occurred in 98 per cent. or over of the quadrats, while the mean number of tillers per quadrat varied from 90.8 for *Agrostis* spp. to 13.8 for *T. repens*. It is seen that the distribution curves are markedly skew. An attempt to fit a binomial distribution to the data for *Agrostis* spp. showed that the curve was not of this type.

Continuous distribution curves. As an alternative method to counting the number of tillers in the quadrat, the percentage area covered by the species can be estimated when the sward is closely grazed. In (Figs. 5-7, distribution curves have been constructed from estimates of the area covered in a sampling quadrat (6 in. x 6 in.). In these distribution curves, the variation is continuous. Examination of Figs. 6-8 shows that the form of these curves is markedly skew.

Fig. 6 shows distribution curves constructed from observations on *Trifolium repens* in four different associations, the areas examined being from 100-200 square metres. In each curve the number of quadrats in the first class is particularly large. It might be thought that this was due to the clover being sparsely distributed so that *T. repens* was absent from a large number of quadrats. While this is true of the curves marked B and C, in curves A and D only 3 and 6 quadrats respectively contained no clover.

In Fig. 7 distribution curves are given for *Agrostis* spp. and *Festuca ovina*, *Agrostis* (A) and *F. ovina* (A) being the dominant and subdominant species in one association, and *Agrostis* (C) and *F. ovina* (B) the dominant species in two other associations. In the case of *Agrostis* A and C, the shapes approximate to normal distribution curves, but those for *F. ovina* do not. The very marked 'skewness' of these two curves cannot be attributed to a sparse distribution, since only 11 quadrats contained no *F. ovina* in the curve marked A and only six in the curve marked B.

In Fig. 8 distribution curves are given for some of the subdominant species in the associations in which the *Agrostis* spp. and *F. ovina* occurred, vide Fig. 6. It is seen that the shapes of these curves are similar to those described earlier. In the case of *Poa trivialis*, *Achillea Millefolium* (A) and *Ranunculus repens*, the large number in the first class can be attributed to the number of quadrats in which no plants occurred, namely 55, 28, and 24 quadrats for the species in question. For *Poa annua*, the shape may in part be due to absence of plants in the quadrat (13 quadrats contained no plants), but for *A. Millefolium* (B) this explanation cannot apply, since from only one quadrat was this absent.

The curves shown in Figs. 5-8 have been chosen as representative of the different forms of the distribution curves. Data were obtained for a large number of other species in the areas examined. As, however, they were in the main those of occasional species, the shapes of the distribution curves were similar to those in which a considerable number of quadrats

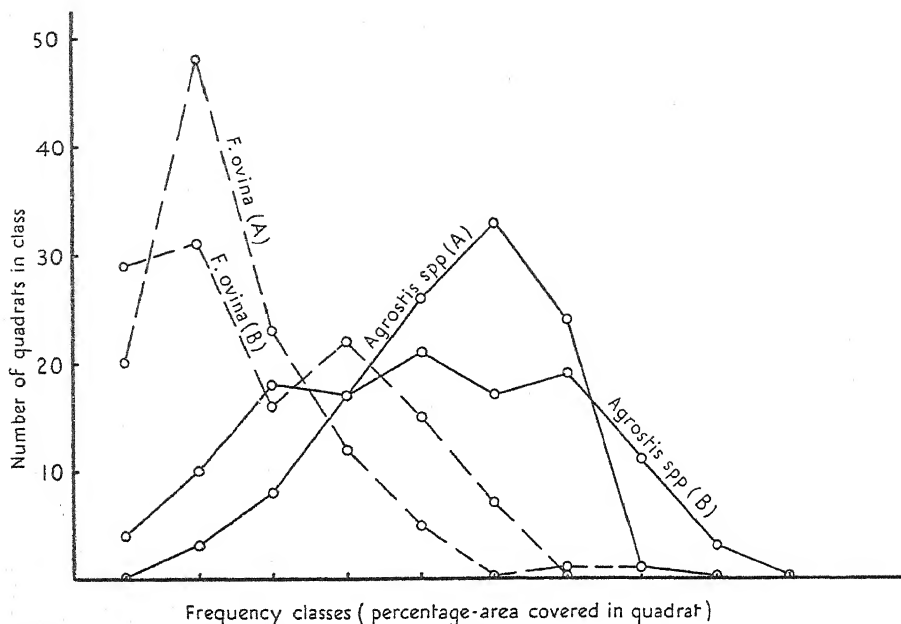


FIG. 7. Distribution curves of density for *Agrostis* spp. and *F. ovina* occurring as dominant or subdominant species in three similar associations. The estimate of density is based on the percentage area covered in the sampling quadrat. The frequency classes are: *Agrostis* spp. A and B, 0-9, 10-19 %; *F. ovina* A, 0-4, 5-9 %; *F. ovina* B, 0-14, 15-29 %. Data collected Jealott's Hill, May 1929, May 1930; Slough, April 1929.

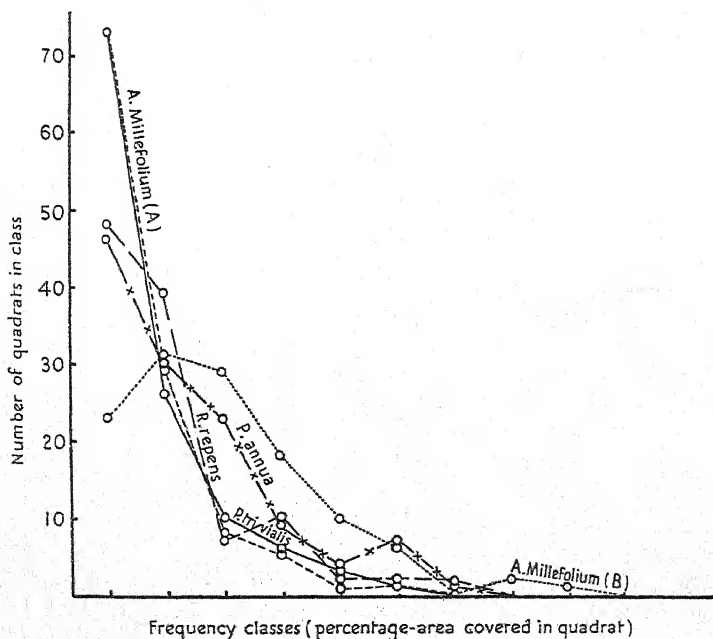


FIG. 8. Distribution curves of density for some occasional species in the three associations observed (vide Fig. 7). The frequency classes in terms of percentage area covered in the quadrat are: *A. Millefolium* (A), 0-7, 8-16 %; *A. Millefolium* (B), 0-2, 3-5 %; *P. trivialis*, 0-9, 10-19 %; *P. annua*, 0-9, 10-19 %; *R. repens*, 0-2, 3-5 %.

contained none of the species, e.g. *P. trivialis* and *A. Millefolium* (B) in Fig. 8. A full discussion of the significance of these distribution curves will be deferred until later in the paper.

Relationship between quadrat size and percentage frequency.

It has already been pointed out that a number of workers investigated purely mathematically the relationship between the size of the quadrat and the average number of species found in it, the calculations being based on an assumed random distribution of the species. Since the evidence brought forward showed distribution curves of a shape which often gave no obvious proof of such a random distribution, it was decided to investigate this relationship.

The sward chosen contained a large number of species. Square quadrats of 2, 4, 8, 16, 32, 64, and 128 square inches in area were used, some thousand quadrats in all being examined on an area of 0.08 acres.

If it is assumed that all the species are distributed at random, then it is possible to calculate the relationship between the quadrat size and the average number of species, provided that the percentage frequencies are known for a quadrat of one size. Let these percentage frequencies be p_1, p_2, p_3 , &c., on the basis that a 100 per cent. frequency is equal to unity. Then the probability that any species a_1 will be found in any given quadrat of size x is p_1 or $1 - (1 - p_1)$. Similarly, the probability for a species a_2 is p_2 or $1 - (1 - p_2)$, &c. Now it can be shown that the average number of species that will occur in any quadrat is

$$\begin{aligned} & p_1 + p_2 + p_3 + p_4 + \dots \\ \text{or} \quad & 1 - (1 - p_1) + 1 - (1 - p_2) + 1 - (1 - p_3) + 1 - (1 - p_4) + \dots \end{aligned}$$

that is to say, for n species the average number found in a single quadrat (\bar{N}_1) will be

$$\bar{N}_1 = n - [(1 - p_1) + (1 - p_2) + \dots + (1 - p_n)] \quad (1)$$

Now if the area of the quadrat is doubled, then the probability that a_1 will occur is $1 - (1 - p_1)^2$ and the average number of species (\bar{N}_2) that will be found in the quadrat ($2x$) becomes for n species

$$\bar{N}_2 = n - [(1 - p_1)^2 + (1 - p_2)^2 + \dots + (1 - p_n)^2]. \quad (2)$$

Similarly, if the quadrat area is again doubled the average number of species found becomes

$$\bar{N}_4 = n - [(1 - p_1)^4 + (1 - p_2)^4 + \dots + (1 - p_n)^4]. \quad (3)$$

It can be seen, therefore, that if the percentage frequencies of all the species in an association have been determined for a quadrat of given size it is possible to calculate the expected number of species as the area of the original sampling quadrat is doubled and redoubled. Formulae similar to the above have been derived by Wickseil (32), Kylin (17), and Arrhenius (2).

TABLE I.
Mean Number of Species on Quadrat.

Size of quadrat. Sq. inches.	Total plot.		Half plot.		Half plot.	
	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.
All species.						
2	4.9	5.9	5.1	5.9	4.7	5.8
4	7.8	7.8	8.0	8.0	7.5	7.5
8	11.3	9.5	11.3	9.8	11.0	9.2
16	15.4	11.1	14.5	11.1	15.1	11.9
32	20.6	13.6	17.4	13.5	19.3	13.5
64	23.5	16.1	20.8	15.7	22.7	17.1
128	26.4	18.2	23.2	17.6	25.0	18.4
Species with frequency of less than 20 per cent. excluded.						
2	4.6	5.5	4.6	5.4	4.1	4.9
4	7.1	7.1	7.0	7.0	6.2	6.2
8	10.1	8.5	9.5	8.1	8.5	7.5
16	13.2	10.1	11.2	9.4	10.7	8.6
32	16.7	11.7	12.3	10.7	12.2	10.2
64	17.4	14.6	12.8	11.8	12.9	11.6
128	17.9	15.6	13.0	12.4	13.0	12.4

In Table I the theoretical relationship between quadrat size and the number of species found in the quadrat is compared with the field data. Figures are given both for the area treated as a whole or divided into two halves. The calculations are based on the percentage frequencies obtained from 400 quadrats of the 4 square inch size in the case of the whole area, or 200 in the case of each half. The upper half of Table I shows that the agreement between the field and calculated figures is not good. It must, however, be borne in mind that with increasing quadrat size the sampling error of the 4 square inch quadrat will be progressively magnified. Inspection of the data indicated that the distribution of the less abundant species was irregular. In the second half of Table I those species with a frequency of less than 20 per cent. in the 4 square inch quadrat have been excluded. It is seen that the agreement between the two sets of figures is considerably improved by the omission of the less common species.

It has been pointed out previously that Arrhenius (2) and Gleason (8) examined in the field the relationship between the quadrat size and the average number of species found in the quadrat. Although details of technique are mostly omitted by Arrhenius it would appear that curves relating to the average number of species and quadrat size were obtained by examining a large number of quadrats of *a single size only*. In order to determine the average number of species found in quadrats of a larger size he grouped the data for the single-sized quadrat at random into sets of 2, 4, 8, &c., quadrats. This method of determining the average number of species in the larger quadrats is open to serious objection. Unless *all* the species are distributed at random the average number determined in this way will not be the same as if quadrats of different sizes had been examined

in the field. For example, if two species are only found in opposite extremities of the area observed, then unless the sampling quadrat is nearly as large as the area under examination, the two species will never occur in the same quadrat. If, on the other hand, small quadrats are grouped at

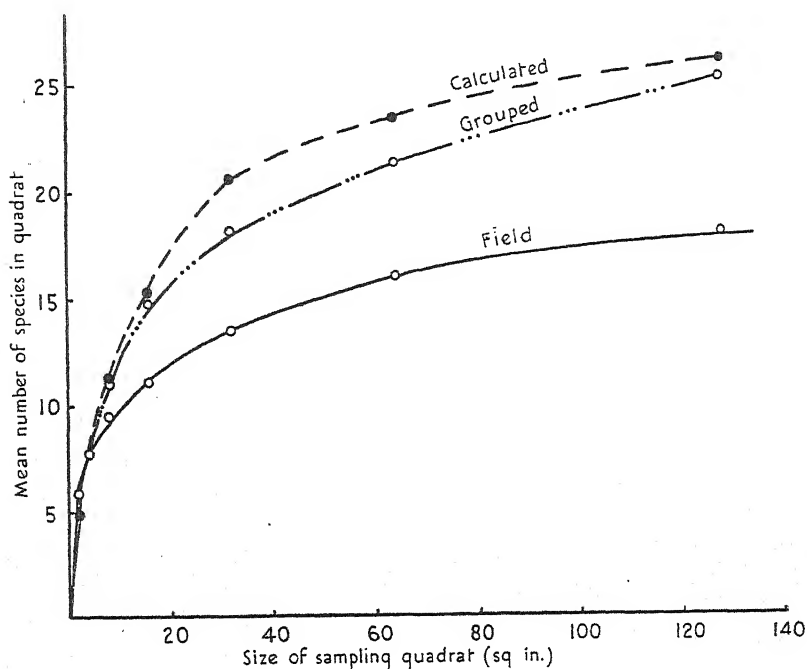


FIG. 9. The relationship between quadrat size and the average number of species found in the quadrat, based on (i) field observations, (ii) calculation assuming a random distribution of all the species, and (iii) grouping the field data for a single-sized quadrat (4 square inches) into sets of 2, 4, 8, &c., quadrats. Data collected Jealott's Hill, August 1932.

random into sets, then it is probable, especially when the number in the set is high, that in quite a considerable proportion of these groupings both species will be found. In fact, the method of grouping tends to even out any irregularity in the distribution of the species. Gleason (9) realized that grouping quadrats at random gave somewhat different results from grouping 'contiguous' quadrats, i.e. quadrats from the same area but not necessarily adjacent. In Fig. 9 a curve is shown relating the quadrat size and the average number of species, the figures being obtained by grouping at random 'contiguous' quadrats (4 square inches) into 2, 4, 8, 16, and 32 quadrats. For comparison the data from Table I for all the species in the whole area have been plotted, together with the calculated curve. It is seen that the curve obtained even by grouping 'contiguous' quadrats is very different from that found in actual practice by observation in the field of different sized quadrats. It is also to be noted that the curve calculated on a random

distribution approaches closely to that obtained by grouping the data. Such an agreement is to be expected, since, as has already been pointed out, the method of grouping tends to nullify unevenness of distribution. It is not, therefore, surprising that Arrhenius (2) obtained in a number of associations so good an agreement between calculations based on a random distribution and data based on groupings *at random* of quadrats of a single size. Such an agreement does not prove that in the associations examined the distribution of the species was at random.

On the basis of the relation between the percentage frequency and the quadrat size, it is possible to test approximately whether a species is distributed at random. If P is the percentage frequency in a square quadrat of area x , then the percentage frequency P^1 in a square quadrat of area y is

$$P^1 = 1 - (1 - P)^{\frac{y}{x}},$$

provided that the distribution is at random.

In Table II a comparison is made between frequencies observed in the field and those calculated on the basis of the above formula. The calculated frequencies for the quadrats of 2, 8, and 32 square inches are based on the data obtained in the field from the 400 quadrats of 4 square inches. Examination of the figures confirms the conclusion already reached, that while the common species tend to be distributed at random, the less common species are not so distributed. It is to be noted that the calculated data give somewhat higher values than those obtained in the field in the case of the larger quadrats, the discrepancies becoming greater as the percentage frequency in the 4-inch quadrat decreases.

TABLE II.
Percentage Frequency.

	4 sq. in.		2 sq. in.		8 sq. in.		32 sq. in.	
	Observed.	Calcu- lated.	Ob- served.	Calcu- lated.	Ob- served.	Calcu- lated.	Ob- served.	
Common species.								
<i>Prunella vulgare</i>	84	60	68	97	99	100	100	
<i>Lotus corniculatus</i>	74	49	57	93	88	100	99	
<i>Trifolium repens</i>	72	47	52	92	87	100	100	
<i>Festuca ovina</i>	67	43	50	89	76	100	99	
<i>Holcus lanatus</i>	50	29	39	75	63	99	90	
Occasional species.								
<i>Luzula</i> sp.	33	18	20	55	49	96	66	
<i>Anthoxanthum odoratum</i>	28	15	7	48	38	93	69	
<i>Cynosurus cristatus</i>	25	14	21	44	34	90	64	
<i>Bellis perennis</i>	18	9	19	33	26	79	49	
<i>Lolium perenne</i>	15	8	9	28	16	73	28	
<i>Poa trivialis</i>	9	5	8	18	14	55	24	
<i>Potentilla reptans</i>	5	3	1	10	7	36	7	

Relationship between percentage absence and density of population.

It has been shown previously that if individuals of a species are distributed at random, then the number of quadrats containing no individuals can be evaluated from the first term of the Poisson series, namely

$$\text{Percentage absence} = e^{-m},$$

where m = the mean number of individuals in the sampling quadrat. Alternatively, e^{-m} can be expressed as e^{-ky} , where k is the density and y the quadrat size. This was the form of the formula given by Kylin (17). *If the percentage absence is known it follows therefore that the density can be calculated.* In Table III the figures for density obtained in the field are compared with those calculated from the percentage absence for the species shown in Figs. 1-4. In addition, the standard error of both the field and calculated values for density have been evaluated from the formulae given in the Appendix, while the differences between the field and calculated values are also shown with their standard errors. The figures have been divided into two sections, those species in which it has been shown that the distribution is of the Poisson type and those species where the distribution is not of this type and is not at random.

TABLE III.

Species.	Density ('plants/sq. ft.')		Difference between calculated and field density.	χ^2 test for Poisson distribution.
	Field.	Calculated.		
<i>Gentiana acaulis</i>	2.60 \pm 0.23	2.44 \pm 0.26	+ 0.16 \pm 0.12	0.8
<i>Arnica montana</i>	3.68 \pm 0.31	3.68 \pm 0.38	0.0 \pm 0.22	0.3
" "	9.80 \pm 0.44	8.64 \pm 0.79	+ 1.16 \pm 0.65	0.8
<i>Primula farinosa</i>	3.12 \pm 0.25	3.12 \pm 0.30	0.0 \pm 0.17	0.7
<i>Primula</i> sp.	2.20 \pm 0.30	2.12 \pm 0.33	+ 0.08 \pm 0.15	0.98
<i>Eryngium maritimum</i>	0.307 \pm 0.038	0.309 \pm 0.045	- 0.002 \pm 0.025	0.9
" "	0.321 \pm 0.038	0.346 \pm 0.049	- 0.025 \pm 0.030	0.8
" "	0.459 \pm 0.046	0.469 \pm 0.061	- 0.010 \pm 0.041	0.8
" "	0.505 \pm 0.041	0.509 \pm 0.060	- 0.004 \pm 0.044	0.5
" "	0.550 \pm 0.048	0.584 \pm 0.065	- 0.034 \pm 0.044	0.95
" "	0.913 \pm 0.053	0.972 \pm 0.108	- 0.059 \pm 0.095	0.5
<i>Primula auricula</i>	9.12 \pm 0.58	5.28 \pm 0.68	+ 3.84 \pm 0.37	< .05
<i>Plantago media</i>	0.624 \pm 0.071	0.464 \pm 0.087	+ 0.16 \pm 0.051	< .05
" "	4.28 \pm 0.24	5.72 \pm 0.30	- 1.44 \pm 0.18	< .05
" "	5.96 \pm 0.28	4.92 \pm 0.36	- 1.04 \pm 0.22	< .05
" "	8.37 \pm 0.33	6.04 \pm 0.44	- 2.33 \pm 0.28	< .05

The figures in Table III show that in the case of species distributed at random, the agreement between the estimated field density and that calculated from the percentage absence is exceedingly good. For species not distributed at random, the agreement between the field and calculated figure is poor in the case of *P. auricula*, but for *P. media* the two sets of figures are of the same order. Since the formulae for determining the

standard errors are based on the assumption that the distribution is of the Poisson type, it would be expected that a χ^2 test for the differences between the field and calculated values of density would show a high value for those species distributed at random and a low value for those species not distributed at random. The value of P for the randomly distributed species in the upper part of the table is 0.8 and for those in the lower part not distributed at random P is less than 0.01.

In view of the fair agreement between the field and calculated density for *P. media*, which is not strictly distributed at random, it was decided to investigate more closely in this species the relationship between percentage absence and density. The data already available showed that the departure from the calculated Poisson distribution was in this species due to too few quadrats containing a single individual and too many 0, 3, 4, and 5 plants. To obtain additional data some 35 areas, each of 150 square feet, were chosen at random in the field; in each area the mean density and the percentage absence were determined by throwing down at random 100 quadrats (6 in. \times 6 in.), the observations totalling in all 3,500. In Fig. 10 the logarithm of the percentage absence has been plotted against the mean density. It is seen that the points fall on a straight line, the regression of the logarithm of percentage absence against the mean density is -0.322 . Since the absolute values of density were not determined, in calculating the regression, it is necessary to take into account that the estimations of density have errors attached. It has been assumed as a rough approximation that the variance of error is proportional to the density and it follows, therefore, that the regression of the logarithm of percentage absence on density can be expressed by the simple formula

$$b = \frac{\text{mean log percentage absence}}{\text{mean density}}.$$

It has been shown that if a species is distributed at random then the logarithm of percentage absence is directly proportional to the density. Since it has been demonstrated that *P. media* is not distributed strictly at random, it is of interest to compare the regression of percentage absence on density already obtained with one based on a random distribution. This regression is shown as a broken line in Fig. 10. It is seen that the two regressions are somewhat different, the discrepancy being due to the distribution of *P. media* not being at random.

In the species so far cited, density has been defined as the number of individuals in a unit area. In the case of species where it is impossible to define an individual plant, it is still possible to obtain a measure of the density by determining either the area covered by the species or the number of tillers in a unit area. If the density obtained in this way is a measure of the number of individuals present, then it would be expected that the

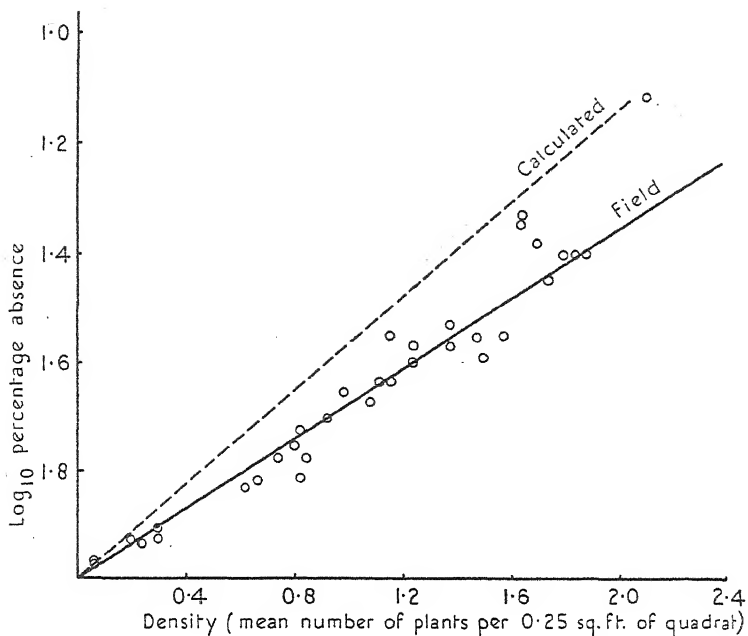


FIG. 10. Graph showing the linear regression of the logarithm of percentage absence on density. The regression calculated on the basis of a random distribution is shown as a broken line. Data collected Cookham, October 1934.

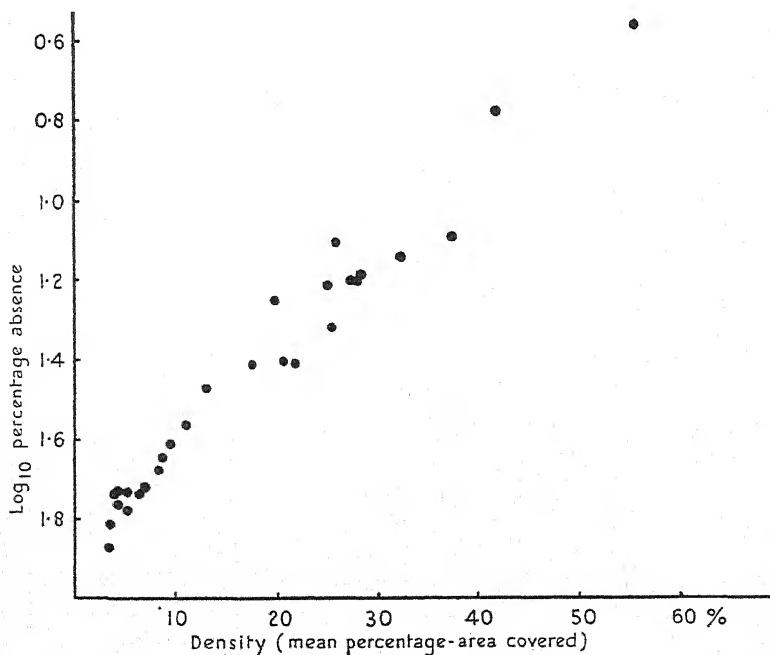


FIG. 11. Scatter diagram demonstrating the correlation between the logarithm of percentage absence and the mean percentage area covered by *T. repens*. Data collected Jealott's Hill, July 1934.

logarithm of the percentage absence would be proportional to the area covered or the number of tillers.

In Fig. 11 the density of *Trifolium repens* as determined by the percentage area covered has been plotted in the form of a scatter diagram against the logarithm of percentage absence. The data was obtained by choosing at random in an association small areas 4 ft. \times 4 ft. The area covered by *T. repens* was estimated by throwing down at random ten quadrats (6 in. \times 6 in.) in each area. The percentage absence was determined from 200–400 small rings (1 cm. diameter), the total number of observations being 6,000.

Fig. 11 shows that there is a marked relationship between the logarithm of percentage absence and density as determined by the percentage area covered. It is to be noted that any fitted straight line would not, however, pass through the origin. Since the logarithm of percentage absence must necessarily be 2.0 when the density is zero it follows that a line in order to pass through the origin must necessarily be slightly curved. The assumption already made, therefore, that the density is proportional to the percentage area covered is not strictly true. It is clear, however, that the error is not large enough to invalidate the conclusion that the logarithm of percentage absence is highly correlated with the density as measured by percentage area covered.

III. DISCUSSION.

In the associations observed, a critical statistical examination has shown for the first time that the nature of the distribution of the species can only be determined by studying (i) the form of the distribution curve, (ii) the relationship between percentage frequency and quadrat size, and (iii) the correlation between the logarithm of percentage absence and density. In this connexion criticism of the work of Arrhenius on the relationship between the number of species and quadrat size has already been advanced.

It has been found for some species that the nature of the distribution cannot readily be deduced from the form of the distribution curve. Where, however, it has been possible to count individual plants, it has been shown that the distribution of a number of species reproduced by seed (*Arnica montana*, *Primula farinosa*, *Primula* sp., *Gentiana acaulis*) is at random, although *Plantago media* is an exception. In species such as *Primula auricula*, where reproduction may occur through short rhizomes, the distribution, as might be expected, was not a random one. On the other hand, in the case of *Eryngium maritimum*, where reproduction may also occur through rhizomes, a chance distribution existed in the areas observed. This difference between *P. auricula* and *E. maritimum* may be due to variation in rhizome length. In *P. auricula* the rhizomes are short and the grouping marked; with *E. maritimum* the rhizomes are much longer

and the grouping is less pronounced. The order of density is also important; when the density is high rhizomes from different parent plants tend to overlap, thus reducing the grouping effect. In species with short rhizomes, this overlapping will not occur unless the density is very high.

In species where individuals can be counted, it has been possible to test statistically for a random distribution. Unexpected difficulties arise, however, in the case of distribution curves based on data of area covered or of tiller counts. It has been seen that the 'skewness' of the distribution curves is pronounced in many cases (*Achillea Millefolium*, *Agrostis* spp., *Cynosurus cristatus*, *Festuca ovina*, *Poa annua*, *P. trivialis*, *Trifolium repens*, *Ranunculus repens*), the degree of 'skewness' being such that mathematical treatment based on the normal bell-shaped type of curve cannot be applied. In many examples the asymmetry of the curves cannot be attributed to a high proportion of quadrats in which the species was absent. On the other hand, a reduction in the number of quadrats in which species are absent has certain mathematical advantages. In continuous distribution curves the limits of variance are theoretically nought and infinity. In the data of this paper the limits of variance tend to become narrowed, while the variance approaches discontinuity in the first classes if a certain number of quadrats do not contain the species. By increasing the quadrat size to such an extent that the species is always present this difficulty can be overcome.

It is interesting to note that similar skew distribution curves were obtained by Morgan and Beruldsen (20) for an Australian pasture. They estimated in a number of small quadrats (3 ft. 11 in. \times 11 in.) the proportion each species contributed to the total dry weight production of the herbage in the quadrat. Although the data offers some difficulty in dividing into equal frequency classes, it would appear that increasing 'skewness' occurred as the density of the species fell. In the four associations described earlier it has already been pointed out that the distribution curves approximating most closely to the normal were those of the dominant species. Morgan and Beruldsen also concluded that the dominant species (*Lolium perenne*) alone appeared to give a normal distribution curve.

To attempt any interpretation of the 'skewness' of these distribution curves is somewhat hazardous on the small number of associations examined. The 'skewness' in many cases is due to too many quadrats with a low density and too few with a high density, when density is expressed as either percentage area covered or number of tillers. Now it can be assumed that the density as measured by area covered or tiller numbers is some function of the number of individuals present in the quadrat, probably, on the evidence for *T. repens* (vide Fig. 11), an approximate direct proportionality. The size of the individual or the number of tillers produced by an individual will be to some extent dependent upon the competition

between species in the association. It would seem probable, however, that the distribution curves of area covered or tiller number must also be an expression of the number of individuals present. If this is so, asymmetrical distribution curves are to be expected. For example, if a number of small, flat, square counters are distributed at random over a plane surface and the area covered by the counters in a sampling quadrat measured, then the distribution curves obtained will be asymmetrical in spite of the distribution being continuous. When the density of counters is low the 'skewness' will be very marked; when the density is high the distribution curve will be of a more normal type. In fact, the distribution curves will approximate in shape to Poisson distributions. This similarity in shape will be governed by the fact that in each quadrat the greater proportion of the area covered in the quadrat must be due to those counters wholly within the quadrat. The probability of the number of whole counters occurring in the sampling quadrat will be expressed by the terms of the Poisson series; it follows, therefore, that the distribution curves of area covered will approximate in shape to Poisson distributions. If instead of plain counters there were used counters with a chess-board pattern of smaller squares, then similar shaped distribution curves would be obtained if instead of measuring the area covered within the quadrat the total number of small squares was observed.

On the above assumption it is to be expected that in the case of the markedly skew curves the density (number of individuals per quadrat) will be low, and conversely with curves of a more normal type the density high. If this is so, then the 'skewness' is some measure of the density. It is therefore to be expected that the dominant species should approximate to a normal distribution curve, and that the 'skewness' would be greatest for occasional species. The curves given in Figs. 5-8 on the whole support such assumptions. The 'skewness' is greatest for the rare species and least for the abundant species. On the hypothesis put forward, however, it is to be expected that in cases where the plant was present in nearly all the quadrats, then the distribution should approach the normal type. In some cases, however (vide Fig. 5), the distribution curves are distinctly skew in spite of 'absence' in the quadrat being less than 2 per cent.

It has already been stated in the introduction that one of the objects of this investigation was to study the distribution of species in grassland associations with a view to determining the best method of estimating changes in the botanical composition of small areas. It has been demonstrated that distribution curves based on area estimations, tiller counts, or yield of dry matter per quadrat, are markedly skew. It has been pointed out that where the species is absent from a large number of quadrats then increasing the quadrat size is advantageous in reducing the asymmetry. In many cases, however, very large quadrats would have to be used, with

the result that the determinations of area covered, tiller numbers or yield per quadrat would be extremely laborious and often quite impracticable. In addition, increasing the quadrat size will lead to a higher personal error in estimations of this nature.

If, however, small quadrats are used, then Fisher's analysis of variance cannot be applied direct to the data, since the theory of this analysis is based on a normal distribution of error. If, however, the form of the distribution curve is sufficiently well known, a suitable transformation to the normal type might be made. It is often possible to treat continuous skew curves by logarithmic transformation. With percentage area covered data $\log. \left(\frac{p\%}{100-p\%} \right)$ might be considered where p = area covered.

It has been demonstrated that where it has been possible to count individuals, the distribution was at random in many cases. In species where it is impossible to count individuals, the nature of the distribution cannot at present be assessed from the distribution curves. On the other hand, the evidence from the relationship between quadrat size and percentage frequency indicates that at least the more abundant species tend to be distributed at random. If the majority of species are distributed at random, then the estimation of percentage frequency becomes of marked ecological importance for determining botanical changes. The greatest advantage is that the logarithm of percentage absence is proportional to the density of the species. This relationship would appear also to hold approximately even when the distribution is not strictly at random, e.g. *P. media*. It follows, therefore, that changes in percentage absence can be directly correlated with changes in density. In addition, the method is liable to little personal error and is rapidly carried out.

If the estimation of percentage absence is used for botanical analysis, the question at once arises as to the order of percentage absence which gives the most accurate measure of density. On the basis of the formula that percentage absence is equal to e^{-ky} it can be shown mathematically that the most accurate results are to be obtained by using a quadrat of such a size that the percentage absence is some 20-30 per cent.¹ The correct sized quadrat is best determined by making a few preliminary observations of percentage absence with any convenient sized quadrat, and then calculating, on the basis of the formula already given (page 762), the size of the quadrat in which an absence of some 25 per cent. should occur.

On the results of the present investigation the value of the conception of a 'constant' species as defined by du Rietz and his co-workers (6) is open to question. It has been shown that a considerable number of species tend to be distributed at random in the association. It follows, therefore, that there is no definite size of quadrat beyond which no further increase

¹ The mathematical proof of this statement is given in the Appendix.

in quadrat size will lead to additional species becoming 'constant', i.e. attaining a frequency of 90 per cent. If the definition of 'minimal area' is modified to denote for *each species* the quadrat size required to obtain a given frequency, then changes in 'minimal area' will be related to changes in density. As it has been seen that a 70-80 per cent. frequency gives the most accurate estimate of density, more trustworthy results for 'minimal area' determinations should be obtained by using this figure rather than the 90 per cent. recommended by du Rietz.

When the percentage absence method of botanical analysis is used the estimation of error presents some difficulty. Where individuals can be counted and the distribution is at random the error can be calculated, since in a Poisson series the variance is equal to the mean. The standard deviation can be obtained from the following formula :

$$\text{Standard Deviation} = \sqrt{\frac{m}{n}},$$

where m = mean number in quadrat, and n the number of quadrats observed. It has been shown that m can be evaluated from the percentage absence figure, since this is equal to e^{-m} . In species where individuals cannot be counted, the estimation of m has purely a hypothetical value, and can only be used on the assumption that the distribution is at random ; proof of such random distribution, it has already been shown, cannot readily be obtained.

It has been demonstrated for a number of species in the grassland associations examined that a close correlation exists between density and the logarithm of percentage absence. It would seem likely that this relationship holds for species in other associations. In this connexion the paper by McGinnies (19) is of interest. McGinnies carried out a large number of observations on the inter-relationship between percentage frequency and density ('abundance'). The areas examined were large (10,000 square metres), while the associations were situated in the semi-desert regions of Arizona. McGinnies found that the correlation between percentage frequency and density ('abundance') was high, but he did not, however, realize the importance of correlating the logarithm of percentage absence and density. In Fig. 12 the relationship between the density and the logarithm of percentage absence has been plotted on the basis of the graph showing the relationship between the figures given for percentage frequency and 'abundance' for unspecified species in Fig. 2 of McGinnies' paper. It is seen that the expected linear relationship is very marked, the regression being -0.1683 . Since McGinnies does not give data in which 'abundance' is stated in terms of the average number of individuals in the sampling quadrat, it is not possible to test how far this regression diverges from that based on a random distribution. Further evidence that this linear relationship exists in other associations has been given recently by Wiehe (33), who

assisted the writer in the field during the course of this investigation. Wiehe showed that in the Aberdovey estuary the density of *Salicornia europea* is proportional to the logarithm of percentage absence.

A modification of the percentage frequency method, known as the 'Point Quadrat' method, is already used for pasture studies in New Zealand.

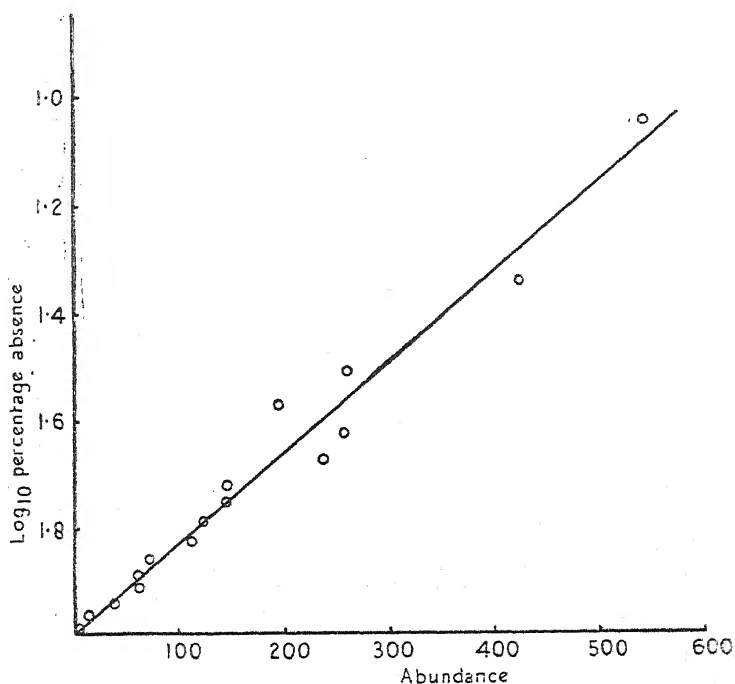


FIG. 12. The relationship between the logarithm of percentage absence and 'abundance' (a measure of density) deduced from published data by McGinnies (1934) for some semi-desert associations.

The method originally suggested by Cockayne (5) has been developed by Levy and Madden (18). It consists in placing at random on the sward a rack holding, in a vertical position, a number of equally spaced steel pins. The pins, having been withdrawn from the rack, are replaced one at a time. As each pin descends, the different species in the sward which come in contact with the pin are noted. The operation is repeated a number of times on the area under investigation, and from the data the percentage frequency determined for each species. Levy and Madden claim that the percentage frequencies obtained in this way can be closely correlated with density as measured by percentage area covered, or composition by weight of the cut herbage. While such a method is suitable for the chief species in the sward, a very large number of observations are necessary for the rarer species. For such species more accurate results

would be obtained by using larger quadrats. In addition, the use of a rack containing several pins has certain disadvantages. For example, if a species occurs as a few isolated clumps, and the rack falls on one of these clumps, all the pins in the rack will fall on the species. In consequence, a biased result will be given unless a large number of readings are taken. More accurate results would be given by using only one pin in the rack. The error of using sets of pins instead of a single pin will be greatest when the pasture is not homogeneous, and least when all the species are distributed at random.

In conclusion it must be borne in mind that these investigations have been confined to a study of small areas only in the associations observed. Although some 12,000 quadrats were examined, the nature of the distribution for many species is still an open question on account of the shapes of the distribution curves. On the other hand, there is evidence that a number of species are distributed at random. Finally, it has been established that the logarithm of percentage absence is proportional to the density for a number of species. The evidence deduced from McGinnies' investigations and Wiehe's results shows that this relationship exists in other widely different associations. Further investigations on other associations will be of great value. At the present stage it would appear that for a detailed study of ecological changes the determination of percentage absence has much to recommend it.

IV. SUMMARY.

In spite of its fundamental importance in quantitative ecology the nature of the statistical distribution of species in an association has not before been subjected to critical analysis. In this investigation the distribution of species over small areas (0.01–0.02 of an acre) has been examined in a number of grassland associations. Some 12,000 quadrats in all were observed.

Where it was possible to distinguish individual plants within the quadrat, it has been demonstrated that a number of species (*Arnica montana*, *Primula farinosa*, *Primula* sp., *Gentiana acaulis*, *Eryngium maritimum*) show a distribution of the Poisson type and therefore a random distribution. Some species (*Plantago media*, *Primula auricula*), however, were not distributed at random. In cases where individual plants could not be determined, e.g. in a stoloniferous species, the density within the quadrat was estimated either on the basis of the area covered by the species or, alternatively, by the number of tillers found. Distribution curves constructed from the data for *Achillea Millefolium*, *Agrostis* spp., *Cynosurus cristatus*, *Festuca ovina*, *Poa annua*, *P. trivialis*, *Trifolium repens*, *Ranunculus repens*, were not of the normal bell-shaped type, but markedly asymmetrical. The 'skewness' of these curves was greatest for the

occasional species and least for the dominant species. An explanation of this 'skewness' is advanced.

In one association the relationship between the quadrat size and the average number of species found within the quadrat was studied. In addition, the theoretical relationship was calculated on the basis that all the species were distributed at random. The agreement between the field data and calculated figures was not very close. The exclusion of the rarer species improved this agreement. Criticism is advanced of previous investigations on this relationship between the number of species and quadrat size.

On the hypothesis of a chance distribution, it was demonstrated that if the percentage frequency of a species is known for a single-sized quadrat, then the frequency in any other sized quadrat can be calculated. Field observations are recorded which in the case of the more abundant species support the formula advanced.

On theoretical grounds it was postulated that if a species is distributed at random then the logarithm of the percentage absence is directly proportional to the density. This relationship was found to exist for a number of species in the associations examined. It was also shown by deduction from the published data of another investigator that this relationship also held for some semi-desert associations in Arizona.

From these observations it is concluded that the estimation of percentage absence in the quadrat has much to recommend it for an ecological study of botanical changes. The method is liable to little personal error, it is rapid, and, above all, changes in frequency can be directly correlated with changes in density. On theoretical grounds, it is shown that the most accurate measure of density is to be obtained by using a quadrat of such a size that the percentage absence is 20-30 per cent.

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APPENDIX.¹

BY

M. S. BARTLETT.

If the distribution of plant numbers is of the Poisson type then the estimate of the density will be

$$\hat{k} = \bar{x}/y, \quad (1)$$

where k is the density, \bar{x} the mean number of individuals found in the

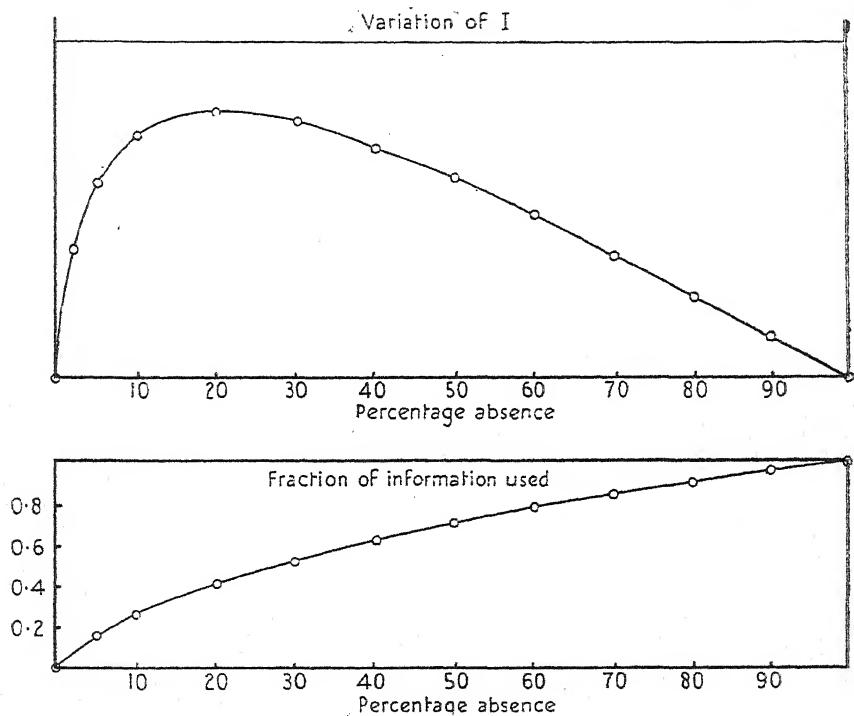


FIG. 13. The upper diagram gives the variation in the information respecting density which is available from noting the percentage absence as the percentage absence changes with size of quadrat. The lower diagram gives the fraction this information is of the total information that would be obtained if the number of plants in each quadrat could have been counted.

quadrat, and y the quadrat size. The standard error of k can be evaluated since

$$\begin{aligned} \sigma^2(x) &= ky \\ \sigma(\hat{k}) &= \sqrt{k/ny}, \end{aligned} \quad (2)$$

¹ Subsequent to obtaining these results, it was noticed that Fisher (7, p. 63) had considered essentially the same problem when estimating the number of organisms in a culture by counting the number of sterile samples. It was thought, however, worth while to derive the formulae used in this paper since the derivation is not given by Fisher.

where n is the number of quadrats observed. The statistical information as defined by Fisher (7, p. 286) is in respect to k

$$I = ny/k. \quad (3)$$

I , as is to be expected, increases with y as well as with n .

When the number of plants within the quadrat is not recorded, but only the absence of the species noted, then it is possible to obtain corresponding equations to those above by differentiation of the 'likelihood function'. A somewhat simpler method of approach in this case is to note that if p is the probability of absence, then the estimate of p will be t/n , where t is the number of times the species does not occur in the quadrat and n the total number of quadrats observed. Now since

$$p = e^{-ky}$$

the equation of estimation for density will be

$$\hat{k} = -\frac{1}{y} \log \frac{t}{n}. \quad (4)$$

Also

$$\sigma^2(\hat{p}) = p(1-p)/n,$$

and for large samples it follows that

$$\begin{aligned} \sigma^2(\hat{k}) &= \sigma^2(\hat{p}) / \left(\frac{\partial p}{\partial k} \right)^2 \\ &= \sigma^2(\hat{p}) / y^2 p^2 \end{aligned}$$

and

$$\sigma(\hat{k}) = \sqrt{(1-p)/npy^2}. \quad (5)$$

The information used is

$$\begin{aligned} I_0 &= npy^2/(1-p) \\ &= ny^2 e^{-ky} / (1 - e^{-ky}). \end{aligned} \quad (6)$$

For different sized quadrats the maximum value of I_0 is given by

$$\begin{aligned} \frac{dI_0}{dp} &= \frac{ny^2}{1-p} + \frac{npy^2}{(1-p)^2} + \frac{2npy}{1-p} \frac{\partial y}{\partial p} = 0, \\ ky - 2(1-p) &= 0, \end{aligned}$$

of which the solution obtained by the method of iteration is

$$\left. \begin{aligned} ky_0 &= 1.594 \\ p &= 0.203 \end{aligned} \right\}. \quad (7)$$

The variation of I_0 is shown in the upper half of Fig. 13. Provided that the size of the quadrat is practicable, it is seen that the estimate of k is most efficient when the percentage absence is 20 per cent. ($p = 0.2$) Since the value of I_0 falls rapidly above $p = 0.2$, it might be advisable in the field to aim at a value of p slightly greater than 20 per cent.

The fraction of information used in the equation of estimation (4) given by

$$I_0/I = pky/(1-p)$$

has been plotted in the second half of Fig. 13. It naturally varies from 0, when there are no cases of absence, to unity, when the quadrat is so small that the cases of absence tend to 100 per cent.

It should be noted that provided the individuals can be counted, equation (1) is a consistent method of estimation whatever the distribution. Equation (4) will only be an exact method when the distribution is of the Poisson type. It may, however, still serve to give comparable approximation values of density if the distribution is only approximately of the desired form (vide Fig. 10), even if the density is defined as a continuous variate such as percentage area covered (vide Fig. 11).

The Quantitative Analysis of Vegetation.

BY

ERIC ASHBY,

WITH AN APPENDIX BY

W. L. STEVENS.

With nine Figures in the Text.

CONTENTS.

	PAGE
I. INTRODUCTION	779
II. HISTORICAL	781
III. EXPERIMENTAL	788
IV. DISCUSSION OF DATA	796
V. SUMMARY	797
VI. APPENDIX	798
LITERATURE CITED	801

I. INTRODUCTION.

IN recent years the basis for the classification and description of plant communities has remained uncertain, notwithstanding the great output of literature on the subject. This literature has had two main concerns: a nomenclature for the units into which ecologists divide vegetation, and the description of vegetation by so-called 'statistical' methods.

It is not the purpose of this paper to discuss nomenclature. Du Rietz (23, 24) has summarized no less than ten systems in use at present, and has ventured to add another, in which the association (as understood in this country) is split into five sub-classes. This extreme subdivision of associations is the logical outcome of the 'statistical' methods adopted by the Uppsala school of ecologists. The object of this paper is to offer a critical review of these 'statistical' and similar methods, and to present some data on the distribution of individuals in a simple plant population.

In a discussion of the 'Individualistic Concept of Plant Association' Gleason (8) distinguished between the spatial arrangement of plants in nature and the definition and classification of such arrangements which exist of course only in the ecologist's mind. At the outset it is useful to

emphasize the distinction raised by Gleason, and to determine precisely on what basis the classification of plant communities is made.

It is clear that in a natural floristic region¹ a limited number of types of vegetation may be distinguished which tend to *recur*, e.g. the beech-woods, moors, and heaths of North-West Europe, and the creosote bush and mesquite of southern Arizona. These recurring plant communities may be accepted as natural units. They are recognized at sight from the physiognomy of the most prominent plants and they have been given popular names without reference to ecology. Yet on closer examination these units are found to be heterogeneous. It is impossible to predict either the exact floristic composition or the spatial arrangement of plants within the community. No one can say what constitutes typical moor and what a departure from the typical, but there is rarely any doubt as to what is a moor.

The incidence of these plant communities is often correlated with some environmental factor; for instance, there is a correspondence between heathland and acid sandy soils in S.E. England. The correlation, however, is not always apparent, and it is evident that the occurrence of a plant community is not determined solely by the environment but depends upon the chances of successful immigration and the stage of succession. In the same way some variations *within* a plant community may be attributed to environmental variation, e.g. the *Sphagnum* in damper parts of a moor, while others, e.g. patches of *Lycopodium*, seem fortuitous in their occurrence. A natural plant community like a moor is well described in Gleason's words (8) as 'representing the selection of a certain environment acting on a given population of species'. Its diversity may be explained on the simple assumption that neither the environment nor the available immigrants are constant over the area.

By the concept of a moor, therefore, is envisaged a sort of mosaic composed of patches of *Eriophorum vaginatum*, of *Vaccinium Myrtillus*, of *Scirpus caespitosus*, of *Calluna vulgaris*, &c. It is the experience of ecologists that while there is general agreement that this community shall be called a moor, there is the greatest difficulty in *delimiting* the 'mosaic-units' which make it up. Is, for example, the floristic composition of the *Calluna*-unit repeated exactly wherever it is found on a moor? In other words, can the moor be regarded as a pattern of rigid units, limited in number, and of the same floristic composition wherever they occur? It is on this point that present evidence seems conflicting. To test the structural uniformity of these units 'statistical' methods have been introduced into the analysis of vegetation.

¹ The expression 'natural floristic region' is introduced here merely for the sake of simplicity. For the present purpose it may be defined as a region over which the plant population available for colonization is roughly constant. Doubtless such floristic regions cannot be sharply delimited, but that is irrelevant to the present discussion.

II. HISTORICAL.

The first quantitative test of homogeneity in vegetation was made by Jaccard (9) who compared the floras of alpine meadows in two apparently simple habitats. Jaccard's method was to evaluate the ratio of the number of species common to both areas to the total number of species in the two areas. This ratio, expressed as a percentage, was called the *coefficient de communauté* (C).

$$C = \frac{\text{number of species common to both populations}}{\text{total number of species in both populations}} \times 100.$$

For two areas of exactly the same floristic composition the value of C is 100, and it tends towards zero as more unlike populations are compared. Jaccard (10) found that the value of C did not exceed about 60 per cent., even when areas apparently similar in floristic composition were compared, and he was therefore led to the conclusion that no two areas ever have exactly the same flora. In a later paper Jaccard (11) tested the agreement between contiguous quadrats of one square metre, within a uniform plant community, and found that the coefficient did not exceed 70.¹

Jaccard's method affords a convenient and simple test of homogeneity between the two populations, but it does not take into account the fact that a species may be rare in one population and common in another. Since the physiognomy of a plant community depends on abundance of species rather than on their mere occurrence, Jaccard's method used alone will not serve for the quantitative analysis of vegetation.

The obvious method of estimating the abundance of a species is to count the number of individuals or to take the weight per unit area, or to measure the area covered. But among plants it is often impossible to distinguish individuals; while the weight method as practised by Stapledon (28), and the area method originated by Shantz (27) are impracticable for many types of vegetation. Considerations such as these led Raunkiaer in 1909 to devise the percentage frequency method (19, p. 393). In this method a quadrat is thrown repeatedly over the plant community to be studied, and the presence or absence of species noted. The percentage of throws in which a species occurs is called its percentage frequency. Raunkiaer adopted a quadrat of 0.1 sq. metre for all his investigations, and usually classified his percentage frequencies into five 'valency' classes: 0-20, 21-40, 41-60, 61-80, and 81-100 per cent. The method gave, according to Raunkiaer, a measure of the 'relative amounts of the species' in any given area (19, p. 307), though Raunkiaer himself realized that the

¹ This value is abnormally high since Jaccard took his data from contiguous quadrats, and there is a positive correlation between the flora of one quadrat and the next. The method applied to British vegetation gives results similar to Jaccard's, e.g. two neighbouring chalk grasslands in Dorset had a ' C ' of 62.2.

method was only an approximate measure of the actual abundance of species (*ibid.*, p. 212). Almost invariably Raunkiaer found that the species were apportioned among the five valency classes in a skew (J-type) distribution, which he expressed conventionally as: $A > B > C \begin{smallmatrix} > \\ < \end{smallmatrix} D < E$ (Fig. 1).

This J-shaped distribution Raunkiaer considered a characteristic of homo-

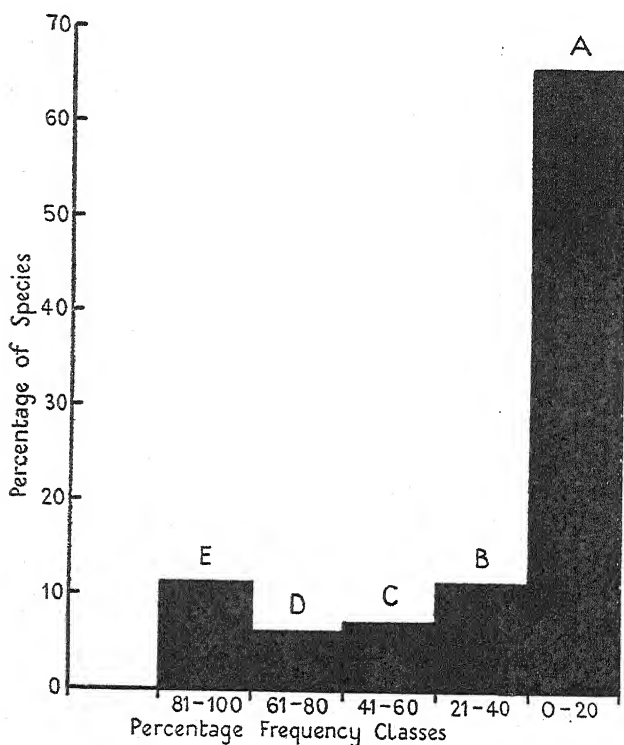


FIG. 1. The distribution of species in percentage frequency classes. Data from Raunkiaer (19, p. 393).

geneous vegetation, and called it the 'law of distribution of frequencies' (19, p. 396).

Since its inception in 1909 the percentage frequency method has been adopted by many ecologists as a rapid and convenient means of summarizing vegetation, and since 1918 it has been considerably elaborated by the Uppsala school of ecologists, whose work is recorded chiefly in the writings of du Rietz. The system of analysing vegetation developed by du Rietz rests upon the assumption that the J-shaped distribution of frequencies is an essential character of a homogenous plant community. Accordingly du Rietz classified species into 'constants' (those occurring in the 90-100

per cent. class), 'occasional' species (those occurring in the 0-10 per cent. class), and a few 'accessory' species in the intermediate classes.

The number of 'constants' will clearly depend on the area of the sampling quadrat; the smaller the quadrat the larger will be the proportion

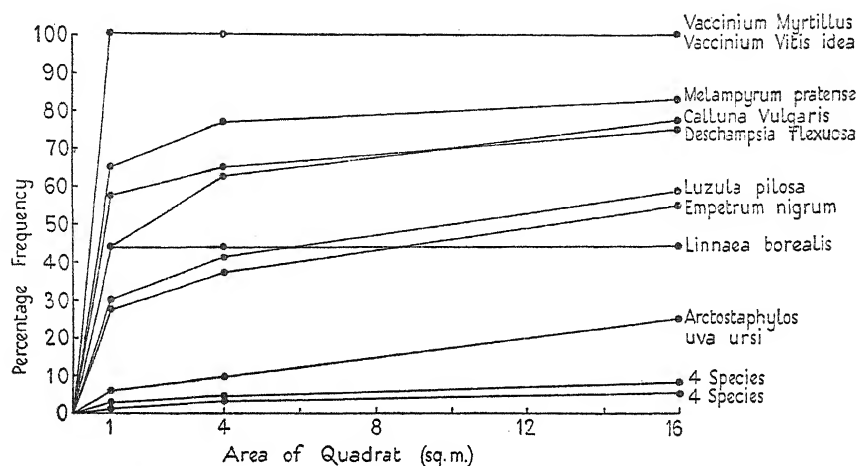


FIG. 2. The relation between percentage frequency and quadrat area.
From du Rietz (21, p. 160).

of species in the 0-10 per cent. class, and the bigger the quadrat the more species will appear as 'constants'. Du Rietz and others (20, 21) collected data as to the relationship between percentage frequency and quadrat size. It was found that increase in quadrat area above a certain size was not accompanied by a corresponding increase in the number of 'constants' (Fig. 2). The quadrat size above which no more 'constants' appear in the community selected was chosen as the 'minimal area'. This area is defined as the 'smallest area on which the association attains its definite number of constants'.¹ The minimal area differs for different communities; it is about one square metre for the moorland vegetation chiefly investigated by du Rietz (Fig. 2). Using a quadrat of the minimal area on such communities du Rietz invariably obtained a J-shaped distribution of frequencies. In fact this distribution is considered to be the test of homogeneity for a community, and on this criterion the Uppsala school maintain

¹ This definition is obviously unsatisfactory, since on the same page (20, p. 35) 'constants' are defined as 'the species of an association which have a percentage frequency over 90 per cent. on an area of sufficient size, i.e. above the minimal area'. In fact, the only definition of a 'constant' in du Rietz's sense is 'a species chosen by the investigator to characterize the community'.

There is some confusion between the minimal area and the more satisfactory concepts of Braun-Blanquet (5) and Palmgren (17). Braun-Blanquet's 'Mindestausmass' is the area above which no new species appear in the community, and Palmgren's 'Minimal Fläche' is approximately the same.

that some of the observations of Jaccard and others were made on heterogeneous populations and not single associations.¹

The general conclusion to be drawn from the writings of du Rietz and his associates is that each of the recurring units of vegetation which together make up a plant community such as a moor is composed of the same definite 'constants' wherever it occurs. This conclusion rests on the fact that when an association¹ is analysed by quadrats of minimal area those species, which by definition characterize the association, occur as 'constants', i.e. with a percentage frequency of more than 90 per cent. It was pointed out by Pearsall (18) in a review of early work by du Rietz, that neither the method of analysis nor the conclusions to be drawn from it are convincing, for the quadrats are not laid at random, but on areas carefully chosen by the investigator. For example, if an *Empetrum nigrum*-*Hylocomium* association is being studied quadrats are laid only where these two species are to be found together. There may be areas where *Empetrum* is accompanied by *Sphagnum* rather than *Hylocomium*, but these are classified as a separate association (21, pp. 160-1). The extent to which these associations differ may be seen from Table I, taken from the data of du Rietz.

TABLE I.

Analysis with One Sq. Metre Quadrat. Empetrum-Hylocomium Association and Two Locí of Empetrum-Sphagnum Association.

Species.	Percentage frequency.		
	<i>Empetrum-Hylocomium.</i>	<i>Empetrum-Sphagnum.</i>	
	(i)	(ii)	(iii)
<i>Andromeda polifolia</i>	4	100	100
<i>Arctostaphylos alpina</i>	10	0	0
<i>Betula nana</i>	100	85	100
<i>Bryanthus coeruleus</i>	1	0	0
<i>Diapensia lapponica</i>	1	0	0
<i>Empetrum nigrum</i>	100	100	100
<i>Leaun palustre</i>	0	0	98
<i>Lycopodium annotinum</i>	34	0	0
<i>Linnea borealis</i>	89	0	0
<i>Oxycoccus macrocarpus</i>	0	98	100
<i>Salix glauca</i>	66	0	3
<i>Vaccinium uliginosum</i>	53	95	100
<i>V. myrtillus</i>	0	0	25
<i>V. Vitis Idea</i>	100	20	68
<i>Equisetum arvense</i>	4	10	0
<i>E. sylvaticum</i>	1	0	23
<i>Pedicularis lapponica</i>	100	0	0
<i>Pinguicula villosa</i>	0	33	93
<i>Rubus Chamaemorus</i>	83	100	100
<i>Calamagrostis lapponica</i>	100	63	30
<i>Eriophorum vaginatum</i>	0	73	100

¹ Association in the sense used by du Rietz until 1927 (24).

From an inspection of this Table there does not appear any obvious reason for separating the two 'associations'. It is true that the second association contains species which do not occur in the first, e.g. *Oxycoccus*, *Eriophorum*, but the two loci of the second association may differ just as strikingly, e.g. *Ledum* and *Vaccinium Myrtillus*. The classification does not seem appropriate unless the percentage frequency is a very sensitive test of the abundance of the species in question. If the two associations are considered as one the J-shaped frequency distribution no longer exists, for there is only one constant—*Empetrum*. It is not clear from du Rietz's writings precisely how samples are taken, though an indication of the method may be found in one reference (24, p. 427) :—'Wenigstens zehn quadratische Probeflächen von bedeutend grösserer Homogenität als derjenigen der Soziation¹ selbst . . . werden sorgfältig ausgewählt . . .' Since these data are not obtained by random sampling, the appearance of 'constants' reflects nothing more than the subjective choice of the investigator. The basis of the method is no more 'statistical' than are the purely descriptive analyses used by British ecologists.

Despite these objections, du Rietz's method might be acceptable if (i) it gave a convenient classification of plant communities, or (ii) if the percentage frequency were a sensitive measure of the abundance or density. As regards classification, the method leads to an extremely inconvenient splitting of plant communities into ill-defined sub-groups. This is evident from the work of two other Scandinavian ecologists, Lindquist and Osvald. Thus in the beechwoods of Scandinavia Lindquist (13) distinguishes thirteen associations in which *Asperula odorata* dominates; *Asperula-Anemone hepatica*, *Asperula-Galeopsis tetrahit*, *Asperula-Laminium galeobdolon*, *Asperula-Melica uniflora*, &c. In a stretch of moorland, five miles by eight, Osvald (16) recognizes no less than 164 associations, besides many 'Assoziationsflecken'. These two examples suffice to show that this classification is worthless from the standpoint of its convenience. It remains to inquire whether the rigidity of 'associations' indicated by percentage frequency analysis depends on the method of analysis or represents real homogeneity in the vegetation. The rest of this section is devoted to this enquiry.

There is no *a priori* reason why the different species in two patches of vegetation should occur in the same proportions; it is in fact surprising to find two communities with five or six 'constants' in common. We have to inquire therefore what character of the vegetation is being measured by the percentage frequency method.

The percentage frequency of a species is simply the chance of finding it in 100 quadrats. As a probability it is valueless as used by du Rietz because the quadrats are not randomly distributed. Du Rietz and his

¹ 'Soziation' is du Rietz's present equivalent of his former 'Assoziation' (24).

associates make the assumption that the percentage frequency is a good measure of the density of species (number of individuals per unit area); although Svedberg (29), Wicksell (30), Nordhagen (15), Kylin (12) and Romell (25, 26) have all pointed out on theoretical grounds that the relation between percentage frequency and density is not linear. Kylin (loc. cit.) in a thorough analysis of the implications of the method, has shown that successive frequency classes (0-10 per cent., 10-20 per cent., &c.) do not represent equal density classes, and that the highest and lowest classes have greater ranges of density than any of the intermediate classes. A demonstration of this fact is worth giving here, since the publications of Kylin are not in English and are somewhat difficult to obtain. The following demonstration is also rather simpler than that due to Kylin.

Consider a large area with relatively few individuals scattered at random over it. Under these conditions the chance of finding 0, 1, 2, 3, ... individuals in a small quadrat will be equivalent to the terms of a Poisson series, e^{-kx} , kxe^{-kx} , $\frac{(kx)^2 e^{-kx}}{2!}$, $\frac{(kx)^3 e^{-kx}}{3!}$... where k is the quadrat size and x the number of individuals per unit area. The probability of *not* finding an individual in a quadrat of area k is therefore:

$$q = e^{-kx} \quad (i)$$

and the probability of finding one or more individuals in the quadrat is

$$1 - q = p = 1 - e^{-kx} \quad (ii)$$

or, expressed as percentages:

$$100 p = 100(1 - e^{-kx}) = \% \text{ frequency} \quad (iii)$$

It is clear that the relation between percentage frequency and density is not linear but logarithmic, and

$$x = -\frac{\log_e q}{k} \quad (iv)$$

The *mean area* of a species, in the sense in which Kylin uses the term, is the reciprocal of the density. Hence if m is the mean area:

$$m = \frac{1}{x} = -\frac{k}{\log_e q} \quad (v)$$

In the following table (Table II) are given the values for percentage frequency with the corresponding density and mean area values, calculated from equation (v) and assuming the quadrat area (k) to be unity.

TABLE II.

Table Calculated from Equation (v) Showing the Ranges of Density and Mean Area Corresponding to the Ten Percentage Frequency Classes. (See also 12, p. 135.)

Percentage frequency.	Density (no. per unit area).	Mean area (= 1/density).	Width of frequency class in mean areas.
90-100	∞ 1-2.303	0-0.434	0.434
80-90	2.303-1.609	0.434-0.621	0.187
70-80	1.609-1.204	0.621-0.830	0.209
60-70	1.204-0.916	0.830-1.091	0.261
50-60	0.916-0.693	1.091-1.443	0.452
40-50	0.693-0.511	1.443-1.958	0.515
30-40	0.511-0.357	1.958-2.804	0.846
20-30	0.357-0.223	2.804-4.480	1.376
10-20	0.223-0.105	4.480-9.488	5.008
0-10	0.105-	9.488- ∞ ²	

It is clear that the division of species into percentage frequency classes gives a distorted measure of the abundance of those species in the vegetation, for in the 90-100 per cent. class are included all species with a mean density of 2.303 or more individuals per quadrat. The values from columns 1, 3, and 4 of Table II are represented graphically in Figs. 3 and 4. The J-shaped distribution, as Kylin and others have pointed out, *does not reflect any peculiarity of the vegetation, but rather of the method of analysis.* Any population which is distributed at random or approximately so will give a distribution which is J-shaped, L-shaped, or U-shaped (according to the quadrat size) if sampled by the percentage frequency method. A normal distribution is never obtained whatever the quadrat size.

It follows that the use of the species in the 90-100 per cent. class as a criterion for homogeneity is unsatisfactory, since this class is the least sensitive as a measure of actual abundance. A species will appear as a 'constant' whether there are three or fifty individuals per quadrat. It is not surprising therefore that the Uppsala ecologists find areas with the same 'constants'. If densities rather than percentage frequencies were measured it is doubtful whether any two patches of vegetation would be alike.

As a means of determining the approximate abundance of species the only frequencies of value are those *below* the 90-100 per cent. class, where the relation between frequency and density is approximately linear, at least over a short range. To sum up, the method of 'constants' cannot be upheld on the grounds of convenience, nor does it provide even an approximate measure of the actual abundance of the common species in a plant community.³

¹ Limited by the area occupied by an individual.

² Limited by the total area of the quadrat.

³ Du Rietz in a recent publication (24, p. 411) presents a method for calculating the 'minimal density' from percentage frequency determinations with different quadrat sizes. The values obtained bear little relation to the actual density and are liable to errors exceeding 100 per cent.

Du Rietz rejects the criticisms of Kylin and others on the grounds that these authors make various assumptions concerning the distribution of species, and have no data to support their assumptions. They assume, for instance, that species are often distributed at random, and they have pro-

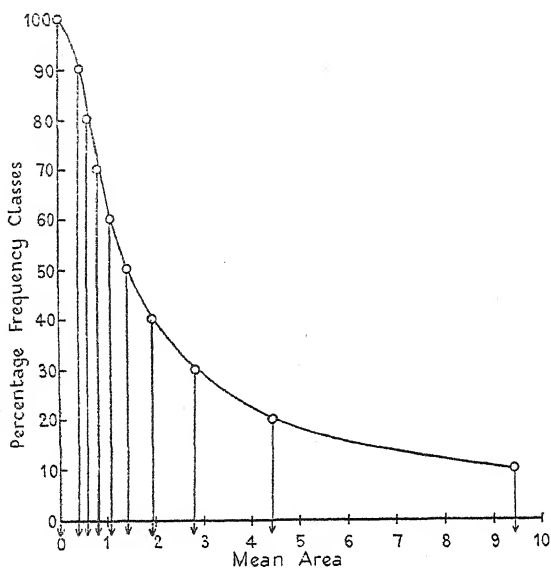


FIG. 3. The theoretical relationship between percentage frequency and mean area on the assumption of random distribution.

vided no practical demonstration of the logarithmic relation between percentage frequency and density. It is true that there is need of data on this point, and accordingly the observations presented below (section III) were made. In the last few months two other papers containing data on this subject have been published: a paper by McGinnies (14) which presents diagrams of percentage frequency and density from plant communities in Arizona but no actual data, and a paper describing many observations on plant distribution in pastures by G. E. Blackman (4). Blackman shows that in pastures most of the common species are distributed at random and the less common species show skew distributions. The frequencies of species distributed at random, or nearly so, do not depart significantly from the terms of a Poisson distribution, so that for these species the relationship between frequency and density is of the kind shown in Fig. 4.

III. EXPERIMENTAL.

In order to determine the relationship between density and percentage frequency in a simple plant population the following observations were

made, which will serve to test the validity of the formulae deduced above (p. 786). The percentage frequency was determined over areas of known density in a population of *Salicornia europaea* in the Dovey estuary.¹ A population of *Salicornia* has many advantages for such an investigation.

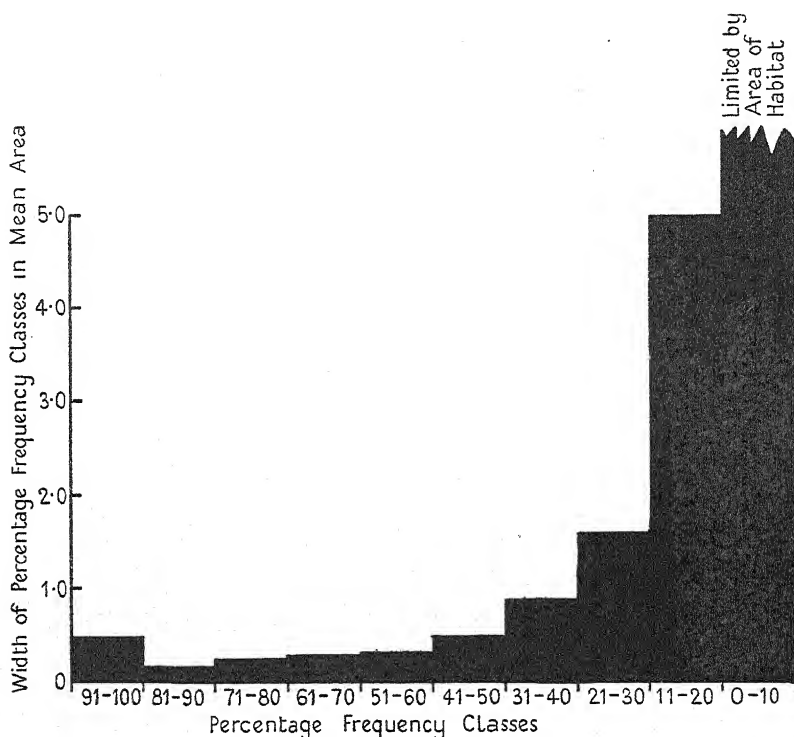


FIG. 4. The range of mean areas corresponding to percentage frequency classes of equal width, on the assumption of random distribution. The figure should be compared with Fig. 1. The differences are due to (i) under-dispersal on account of competition, and consequently more species in 91-100 per cent. *F.* class, and (ii) the fact that the number of species is limited, and therefore the 0-10 class is not really infinitely large.

Wiehe (31) has shown that the density varied from 2 to 700 individuals per square metre, and small areas of uniform density are common. The size of the plants is small compared with their mean area, and there is no difficulty in distinguishing one individual from another. Apart from the tidal zones (31) the environment is homogeneous, and there is no complication arising from the competition of other species. Such a population provides excellent conditions for an initial study of plant distribution.

Two quadrat sizes were used, and the data were collected from about 4,000 quadrats. The procedure was as follows: *Series (i)*. One hundred squares of 625 sq. cm. were taken at random. Each square was subdivided

¹ I have to thank Mr. P. O. Wiehe for collecting these data.

into 25 quadrats, each 25 sq. cm. in area. The average density of *Salicornia* in each square was determined by counting the number of individuals, and at the same time the presence or absence of individuals in each of 25 quadrats was noted. In this way a value for percentage frequency corresponding to the density of every square was obtained, i.e. 100 values of frequency-density. *Series (ii).* The procedure was repeated in another series of 60 larger squares, each 2,500 sq. cm. in area, subdivided into 25 quadrats of 100 sq. cm. area. In this way two series of frequency-density values were obtained, the first for a quadrat of 25 sq. cm., the second for a quadrat of 100 sq. cm. The data are given in Tables III and IV.

TABLE III.

Number of Individuals of Salicornia Europea in Squares 625 sq. cm. in Area, together with Percentage Frequencies from Quadrats 25 sq. cm. in Area laid Twenty-five Times in Each Square.

N. = number of individuals per square.
% F. = percentage frequency (from 25 observations).

<i>N.</i>	<i>% F.</i>	<i>N.</i>	<i>% F.</i>	<i>N.</i>	<i>% F.</i>	<i>N.</i>	<i>% F.</i>	<i>N.</i>	<i>% F.</i>
76	96	55	88	11	32	48	68	17	44
28	56	70	86	43	72	50	84	82	80
100	100	104	96	47	68	52	80	20	52
23	56	52	84	37	80	8	28	3	12
5	20	14	32	50	20	4	12	3	12
2	8	61	88	73	84	75	84	91	92
96	92	110	96	30	68	20	44	12	20
26	68	65	96	40	88	9	36	51	88
49	88	45	76	77	96	23	44	82	100
51	80	78	100	28	68	18	48	13	24
12	28	6	24	4	16	5	16	68	92
68	100	88	100	127	100	132	100	133	100
39	80	91	100	68	96	61	92	14	32
29	60	64	92	50	88	67	100	69	96
74	100	50	76	53	88	42	76	79	100
7	28	117	100	29	60	22	48	28	68
39	76	70	96	60	84	40	68	36	76
54	88	34	56	63	88	31	76	29	68
31	72	73	92	62	80	52	88	78	92
17	32	5	20	3	12	11	20	5	16

In Fig. 5 the frequencies are plotted against corresponding densities. It is obvious that the relation between these two variables is not linear. The observations confirm the prediction made by Kylin (12): that there is a much greater range of densities corresponding to the upper than to the lower frequency classes. Thus, when the 100 cm. sq. quadrat is used all densities from 2.32–9.4 individuals per quadrat fall in the 90–100 per cent. class, while the 80–90 per cent. class has only a tenth of this density range, 1.52–2.32 individuals per quadrat.

If the relation between frequency and density is logarithmic the values of density (x) plotted against $\log_e q$, where q is the chance of *not* finding a

plant, should fall along a straight line (see formula iv, p. 786). Reference to Figs. 6 and 7 shows that this is approximately true. The points are grouped around the theoretical line calculated from formula iv. It may be concluded therefore that the distribution of individuals of *Salicornia* is

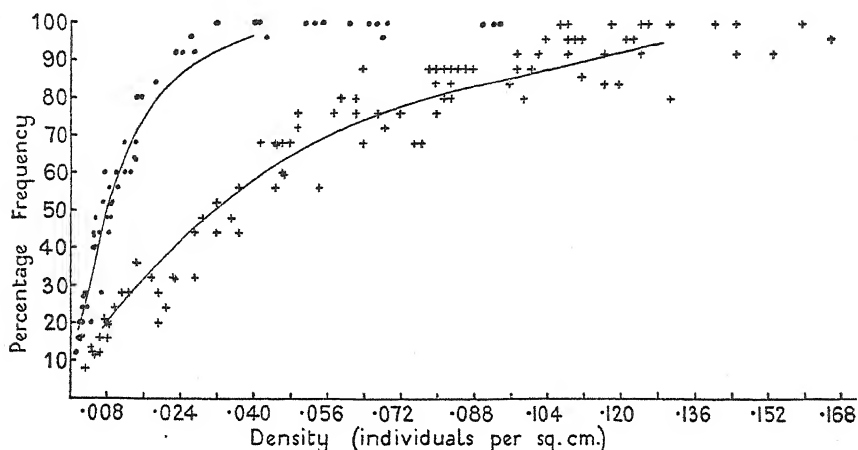


FIG. 5. Percentage frequency plotted against density in individuals per square centimetre for a population of *Salicornia*. The dots represent observations taken with a quadrat 100 square cm. in area, and the crosses represent observations taken with a quadrat 25 square cm. in area.

roughly random, and therefore the relation between frequency and density is logarithmic rather than linear. The criticisms made on this assumption against the methods of du Rietz and his associates are accordingly justified by these data.

TABLE IV.

Number of Individuals of Salicornia Europea in Squares of 2,500 sq. cm. in Area, together with Percentage Frequencies from Quadrats of 100 sq. cm. in Area laid Twenty-five Times in Each Square.

N = number of individuals per square.
% F. = percentage frequency (from 25 observations).

<i>N.</i>	% F.	<i>N.</i>	% F.	<i>N.</i>	% F.	<i>N.</i>	% F.	<i>N.</i>	% F.
39	80	38	80	47	84	66	96	30	60
23	52	15	40	139	100	235	100	232	100
129	100	152	100	69	92	171	96	61	92
58	92	101	100	104	96	173	100	225	100
12	40	14	40	25	60	16	44	13	44
5	16	17	28	6	24	13	44	9	28
21	44	19	60	26	56	8	28	21	56
22	48	35	64	35	64	102	100	16	48
21	48	30	68	33	60	5	20	3	12
4	16	8	24	4	16	11	20	5	16
5	16	5	20	7	28	18	52	36	68
80	100	22	52	135	100				

In order to test the agreement between the observed values and the theoretical lines in Figs. 6 and 7 regression equations would have to be calculated. This necessitates weighting each value according to its variance, and is a very laborious process. A more sensitive test of random distri-

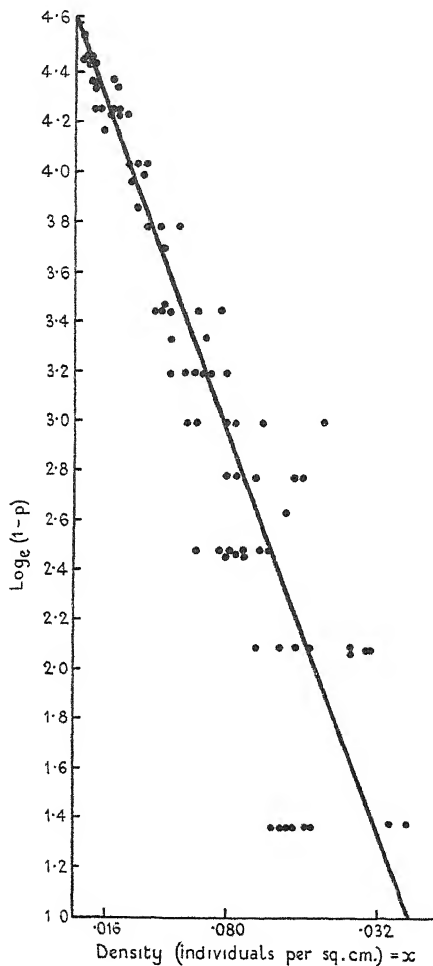


FIG. 6. Natural logarithms of the chance of *not* finding an individual in a quadrat 25 square cm. in area, plotted against density of *Salicornia* in individuals per square cm., together with the theoretical line on the assumption of random distribution.

bution has been suggested to the writer by Professor R. A. Fisher and Mr. W. L. Stevens. A description by Mr. W. L. Stevens of this method will be found in an appendix to the present paper. The operation of the test is as follows:

If the individuals in a population are distributed randomly and it is sampled by the method described on p. 790, the expected number of empty

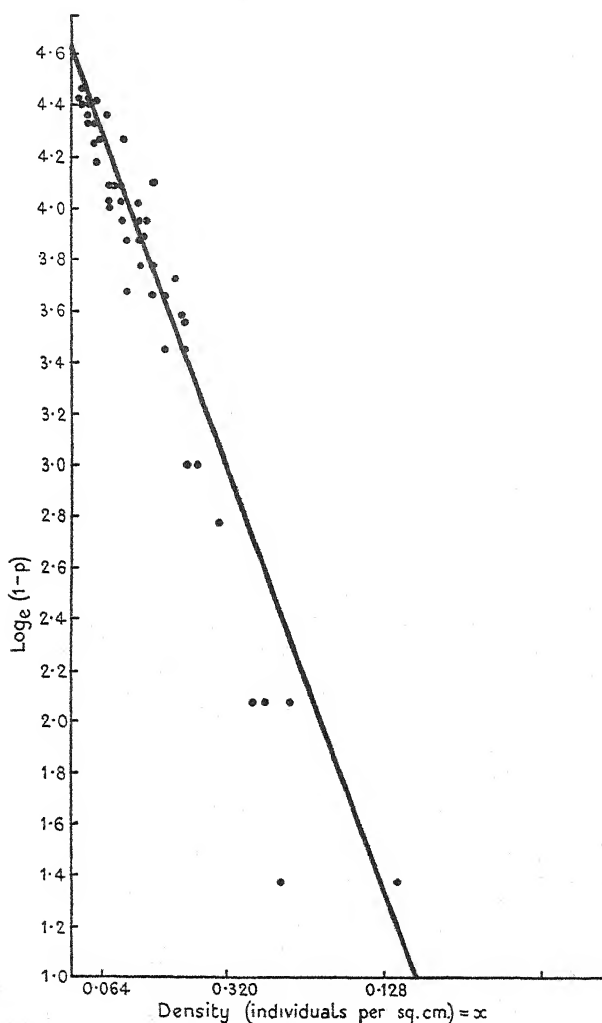


FIG. 7. Natural logarithms of the chance of *not* finding an individual in a quadrat 100 square cm. in area, plotted against density of *Salicornia* in individuals per square cm., together with the theoretical line on the assumption of random distribution.

quadrats (quadrats with zero frequency) in a sample square is given by E , where

$$E = n \left\{ \frac{n-1}{n} \right\}^s \quad (\text{vi})$$

where n = the number of quadrats into which the square is subdivided (in these experiments $n = 25$) and s = the number of individuals per sample

square. The variance of the observed number of empty quadrats for each square is

$$v = n \left\{ (n-1) \left(\frac{n-2}{n} \right) - n \left(\frac{n-1}{n} \right)^2 + \left(\frac{n-1}{n} \right)^2 \right\} \quad (\text{vii})$$

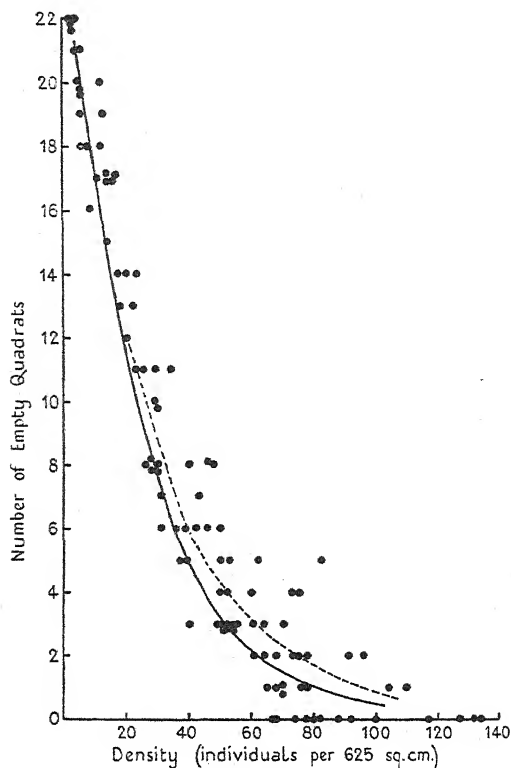


FIG. 8. Number of empty quadrats in a lattice of 25 thrown on 100 areas of known density, plotted against the density, together with the theoretical curve. Quadrat size, 25 square cm.

In Figs. 8 and 9 the number of empty quadrats is plotted against the corresponding density. The theoretical line is calculated from equation (vi). Distribution is clearly approximately random; but it will be noticed that most of the points lie *above* their theoretical values, i.e. there are consistently *more* empty quadrats than would be expected on the assumption of random distribution. The significance of these departures from the calculated line may be tested by summing the observed values of E and subtracting this from the sum of the calculated values of E (Table V, opposite).

In each instance the differences are more than four times the standard deviation. This indicates that there is significant under-dispersion or aggregation among the individuals of the population. The amount of aggregation may be estimated by applying a correction to equation (vi),

from which a curve is then calculated which more closely fits the data. The method of making this correction is due to Mr. W. L. Stevens, and is described in the Appendix. The modified equation is :

$$E = n \left(\frac{n-1}{n} \right)^s \{ 1 + s(s-1)c \} \quad (\text{viii})$$

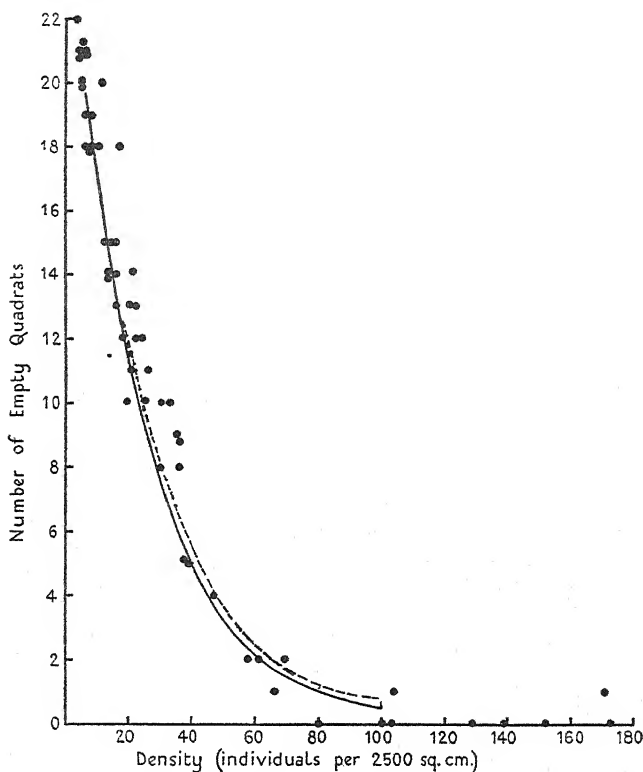


FIG. 9. Number of empty quadrats in a lattice of 25 thrown on 68 areas of known density, plotted against the density, together with the theoretical curve. Quadrat size, 100 square cm.

where c is the correction for heterogeneity. The fitted curves are shown by the dotted lines in Figs. 8 and 9, and the values of ' c ' for the two quadrat sizes are 11.72×10^{-5} and 12.3×10^{-6} . These values are a measure of the degree of aggregation for the populations.

TABLE V.

Test of Significance of Departures from Theoretical Lines in Figs. 8 and 9.

Quadrat size in sq. cm.	Sum of number of empty quadrats		Sum of differential variances.		σ .
	obs.	cal.			
25	754	671,725	82,275	135,066	11.61
100	610	568,250	41,250	72,757	8.53

The cause of this slight under-dispersion is obscure. It might be due to characteristics of the environment, e.g. differential soil fertility, or to peculiarities of the species, e.g. non-random seed dispersal. Further work is needed before any hypothesis can be offered.

IV. DISCUSSION OF DATA.

The observations just presented show, for the simple conditions of a population of *Salicornia* on a mud flat, that the individuals are distributed practically at random within a uniform environment, with a slight but significant aggregation. Under these conditions the relation between density and percentage frequency is approximately logarithmic. The data are completely in accord with the theoretical conclusions presented in the earlier part of this paper, which may be summarized under the following three heads:

1. The percentage frequency of a species gives a distorted measure of its density (individuals per unit area) since equal classes of frequency do not correspond to equal classes of density. It is impossible, therefore, with the technique used by du Rietz, to deduce the density from the percentage frequency.

2. The J-shaped distribution of species in percentage frequency classes does not indicate any exact numerical relationship among the species composing a community, nor is it any indication of homogeneity. It is merely consequent upon the fact that the lowest and higher frequency classes may embrace wider classes of mean area than the intermediate frequencies (Table II, col. iv).

3. The 90–100 per cent. class includes all species whose density is greater than 2,303 individuals per quadrat. It is the least sensitive part of the frequency scale; accordingly the ‘constants’ of the Uppsala school may vary widely in density.

The data presented in section III have purposely been taken from the simplest kind of population, where individuals could be distinguished, and where no correction need be applied for the size of plants. Even so, there is a slight amount of aggregation, the magnitude of which cannot be predicted on theoretical grounds. That the same sort of result is given by data from more complex communities appears from G. E. Blackman's work on pastures (4). If the methods of du Rietz and his associates are inapplicable to these communities they are even less applicable to more complex communities where environment, size of plant, and stage of succession all vary.

The criticisms brought against the ‘statistical’ methods of the Uppsala ecologists are therefore fully supported by such experimental evidence as has been collected. Raunkiaer used the percentage frequency method as a rapid and approximate way of describing vegetation, and as such it may

be upheld. But as a basis for classification of communities the method is justified neither by convenience nor by the correctness of its basal assumptions. In determining his 'constants' the investigator simply makes a personal judgement as to the limits of the association. In this judgement lies the essential classification. Any subsequent 'statistical' analysis of the kind described must inevitably confirm the investigator's choice. Furthermore, the artificial delimitation of communities consequent upon 'statistical' analysis (e.g. the examples quoted on p. 785) is misleading, since many of the associations described by the Scandinavian ecologists are clearly serial stages of succession.

Finally, it seems that the application of quantitative methods to plant communities has not shown that the constituent units of vegetation have any consistent 'make-up'. Both a moor and patches of *Calluna* on a moor are recognized by common physiognomic criteria and are classified subjectively. There is no evidence of a definite internal spatial arrangement of species corresponding to this classification.

Although quantitative methods fail when applied to the classification of plant communities, statistical methods are proving most profitable for studying the distribution of *individual species* within the community. The work of G. E. Blackman has shown how the methods can be applied to a study of the changes in pastures following grazing or manurial treatment; and the technique outlined in section III of this paper can be used to test the degree of dispersion of a species and the changes in distribution during successional history.

V. SUMMARY.

A critical discussion is given of the application of quantitative methods to the analysis of plant communities. Reference is made to the work of Jaccard and Raunkiaer, and the mathematical basis of the percentage frequency method is examined. A review of recent work by the Uppsala school of ecologists leads to the conclusion that the analysis of vegetation based on 'minimal areas' and 'constants' rests on unsatisfactory assumptions and results in a misleading classification of plant communities.

To test the validity of objections to the 'minimal area' method made on theoretical grounds, density and frequency determinations were made on a population of *Salicornia europea*. Data from 4,000 quadrats support the theoretical discussion, and show that—

(i) the individuals are distributed almost at random in a uniform environment; there is a small but significant under-dispersion (aggregation) of the individuals,

(ii) the amount of aggregation may be estimated by fitting the equation

$$E = n \left(\frac{n-1}{n} \right)^s \{ 1 + s(s-1)c \}$$

to the data, where E = the number of empty quadrats in a sample lattice divided into n quadrats, s = the number of individuals in a sample lattice, and c = a correction applied for aggregation of individuals (see Appendix),

(iii) the relation between percentage frequency and density is given approximately by the equation $p = 1 - e^{-kx}$, where p is the probability of finding a species of density x in a quadrat of area k ,

(iv) support is given to Kylin's suggestion that the J-shaped skew distribution of species in percentage frequency classes depends on the fact that frequency classes of equal width do not correspond to equal density classes.

It is concluded that—

(i) the classification of plant communities depends on subjective criteria, e.g. physiognomy, and the application of quantitative methods has not facilitated classification,

(ii) there is no evidence that the recurring patches of vegetation which compose a plant community are consistent in their internal floristic composition,

(iii) the value of statistical methods in the analysis of vegetation lies in their application to the distribution of individual species within the community, not in the analysis of the community as a whole.

I have pleasure in thanking Professor R. A. Fisher and Mr. W. L. Stevens for suggestions as to the mathematical treatment of the results, Professor V. H. Blackman and Dr. F. G. Gregory for their criticisms of the subject-matter of the paper, and Mr. P. O. Wiehe for collecting the observations on *Salicornia*.

APPENDIX.

THE RELATION BETWEEN PLANT DENSITY AND NUMBER OF EMPTY QUADRATS.

BY

W. L. STEVENS.

(*Galton Laboratory, University College, London.*)

(A) *Random distribution.*

The following notation will be used :—

Number of quadrats into which the square is divided = n .

Total number of individuals inside the square in any trial = s .

Number of empty quadrats in this trial = e .

The mean, or expected, value of any quantity in random samples will be denoted by placing a line over the quantity, thus: \bar{e} .

If the distribution inside a square is random, then a single individual is equally likely to fall in any quadrat, independently of the distribution of the remaining individuals. Since there are n quadrats, the probability of an individual falling in any quadrat is $\frac{1}{n}$ and the probability of its not falling in the quadrat is $1 - \frac{1}{n}$. Consequently the probability that the quadrat will be empty is

$$p = \left(1 - \frac{1}{n}\right)^s$$

The expected number of empty quadrats is the sum of these probabilities over all quadrats in the square.

$$\bar{e} = n \left(1 - \frac{1}{n}\right)^s \quad (1)$$

Now let $p_{\lambda\mu}$ denote the probability that quadrats λ and μ are simultaneously empty. Then, if there are e empty quadrats,

$$\frac{e(e-1)}{2} = \text{the number of pairs of quadrats contained in the } e \text{ empty quadrats.}$$

Hence every pair of empty quadrats contributes unity to the total $\frac{e(e-1)}{2}$ and the expected value of $e(e-1)$ is given by

$$e(e-1) = 2 \sum p_{\lambda\mu}$$

where the summation proceeds over all pairs λ and μ .

But the probability of an individual falling outside two specified quadrats is $\left(1 - \frac{2}{n}\right)^s$ and hence

$$p_{\lambda\mu} = \left(1 - \frac{2}{n}\right)^s \text{ for all } \lambda, \mu,$$

$$\text{and} \quad \overline{e(e-1)} = n(n-1) \left(1 - \frac{2}{n}\right)^s \quad (2)$$

The left-hand side of equation (2) may be written $\overline{e^2} - \bar{e}$.

Hence the variance may be obtained by adding \bar{e} and subtracting $\overline{e^2}$; i.e.:

$$\text{variance } (e) = n \left\{ (n-1) \left(1 - \frac{2}{n}\right)^s + \left(1 - \frac{1}{n}\right)^s - n \left(1 - \frac{1}{n}\right)^{2s} \right\} \quad (3)$$

It is now possible to test whether the data show significant departure from the random distribution theory. For each trial, the observed value of s enables us to find \bar{e} and $\text{var. } (e)$. Since the trials are independent, the means and variances may be summed over all trials, to give the expected total of empty quadrats and the variance, and hence the standard deviation of the total. Although the distribution of e is far from normal, the sum

for a considerable number of trials approximates sufficiently to normality, to permit the use of the usual test of significance.

(B) *Non-random distributions.*

There are of course any number of theoretical distributions showing progressive departure from randomness, but we shall discuss one which can be derived from a very simple assumption. Let us suppose that the probability of an individual falling in a quadrat varies slightly for the different quadrats, but that this probability is still independent of the distribution of the remaining individuals. Such conditions would arise from a variability in environment, e.g. soil fertility, over the square.

The probability of an individual falling in quadrat λ is now $\frac{1}{n} + \epsilon_\lambda$

where ϵ_λ is small compared with $\frac{1}{n}$.

$$\sum \left(\frac{1}{n} + \epsilon_\lambda \right) = 1$$

and therefore

$$\sum \epsilon_\lambda = 0$$

The probability of quadrat λ being empty is now

$$p_\lambda = \left(1 - \frac{1}{n} - \epsilon_\lambda \right)^s$$

and the expected number of empty quadrats is

$$\begin{aligned} \bar{e} &= \sum p_\lambda = \sum \left(1 - \frac{1}{n} - \epsilon_\lambda \right)^s \\ &= \sum \left(1 - \frac{1}{n} \right)^s + s \sum \left(1 - \frac{1}{n} \right)^{s-1} \epsilon_\lambda + \frac{s(s-1)}{2} \sum \left(1 - \frac{1}{n} \right)^{s-2} \epsilon_\lambda^2 + \dots \\ &= n \left(1 - \frac{1}{n} \right)^s + \frac{s(s-1)}{2} \left(1 - \frac{1}{n} \right)^{s-2} \sum \epsilon_\lambda^2 \text{ approximately} \\ &= n \left(1 - \frac{1}{n} \right)^s \{ 1 + s(s-1)c \} \end{aligned} \quad (4)$$

$$\text{where } c = \frac{\sum \epsilon_\lambda^2}{2n \left(1 - \frac{1}{n} \right)^2}$$

The constant c , which may be assumed to be the same for all trials in a limited area, is a measure of the tendency of individuals to occur in clumps, and is equal to zero for the random distribution previously considered.

A thorough investigation of the agreement between the data and the above theory, and the efficient estimation of the constant c , would require a knowledge of the exact distribution of e . This is outside the scope of the paper, and it is proposed merely to find a rough estimate of c , in order

to draw a corrected curve (Fig. 9), and to judge by eye whether this curve appears to fit the data.

The crudest estimate would be obtained by equating the observed total number of empty squares to

$$\sum n(n-1)^s \{1 + s(s-1)c\}$$

summed over all trials and solving for c . But we know that the variance of e depends on s . Consequently a better estimate is obtained by sub-totalling e and \bar{e} over groups, and weighting these subtotals with the reciprocals of their variances. These variances of course are functions of c , but if c is small we may use the variances previously obtained on the assumption of random distribution.

For a full discussion of the distribution of e the reader is referred to a paper which will shortly appear in 'The Annals of Eugenics'.

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An Inexpensive Recording Porometer.

BY

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AND

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With six Figures in the Text.

DURING an investigation of the water relations of seedling tomato plants a record of the stomatal movements over a period of twenty-four hours was required. The very expensive clockwork drum of the type used to obtain a record covering this period with a time interval of less than five minutes was not available. The apparatus described below was used in its place with highly satisfactory results. Slight modifications were also made in the electrical bubble counting device originally described by Knight (1).

The improvement on Knight's bubble counter consisted in fitting a 'constant level' supply to the water vessel, thus increasing its effective capacity without introducing increased sensitivity to thermal changes. An additional advantage was the extreme ease with which the water reservoir could be refilled without disturbing any of the delicate adjustments. Fig. 1 shows Knight's bubble counter fitted with the constant level attachment, and is almost self explanatory. Some little difficulty was found at first in avoiding irregular action due to surface tension of the water in the tube A, but this was overcome by using a tube having an internal diameter of not less than 8 mm., bent and cut off as shown in the sketch.

In place of the clockwork drum, a flat sheet of copper, on which was pasted the paper to take the record, was used, the record being obtained by perforations produced by the spark of a small Ruhmkorff coil. In the actual construction, several points of detail were found to be important.

Fig. 2 shows the complete apparatus. The clock (A) was placed in the centre of the copper plate (B), and insulated from the plate by a glass disc (C). To the hour hand of the clock was affixed a metal arm (D) which carried a

small trailing copper wire (F) (Fig. 3). Each bubble passing through the vessel E caused a spark to pass from the wire trailer on the clock, through the paper to the copper plate. By bending the minute hand of the clock into a vertical position it was an easy matter to obtain contact between coil

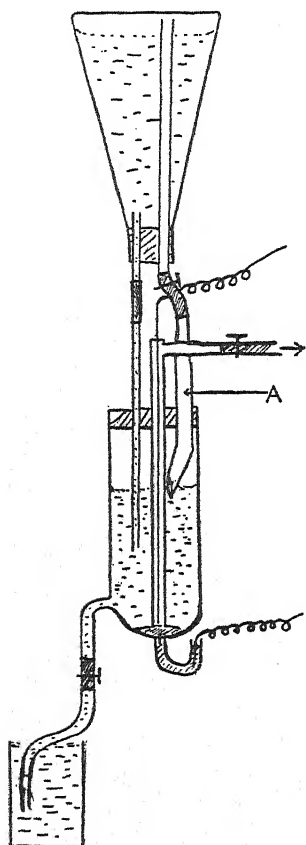


FIG. 1. Knight's porometer with 'constant level' attachment.

and clock without interfering with the movement of the hour hand and its arm. To obtain a twenty-four hour record with a twelve-hour clock, it was necessary to use two wire trailers consecutively on the hour-hand arm, giving two tracks on the paper chart about half an inch apart. One trailer (F) was removed from the arm at midnight by a simple trip device shown in Fig. 3, and at the same time the second trailer (G) was automatically picked up by the hour-hand arm (H). The second trailer (G) was placed so as to hang vertically from the tip of the wire support (K), the tip being directed tangentially, and pointing in the direction of rotation of the arm, so that the hook of the trailer would catch in the groove (L) of the arm (H) as the latter passed the support (K).

At first an attempt was made to utilize the spark obtained from a coil and two 2-volt accumulators connected with the bubbler, but the single impulse produced by the escape of one air bubble did not produce a spark of sufficient duration to make a distinct mark on the paper. To overcome this difficulty, the porometer was connected in series with one accumulator and the electro-magnet and armature of an electric bell mounted on its side. The bell itself was removed, and about 9 in. of thin galvanized iron wire was attached to the hammer. A thin copper wire attached at right angles to the end of the iron wire made contact with a pool of mercury when the electro-magnet of the bell was actuated. When the hammer arm came into contact with the mercury the battery-coil-clock circuit was completed, as shown in Fig. 4, and the spark from the secondary of the coil passed through the paper. The flexibility of the iron wire on the bell hammer was such that for each impulse actuating the electro-magnet four or five contacts were made in rapid succession with the mercury, and this number of sparks passing from the coil was sufficient to make a visible perforation of the paper chart. To protect the apparatus a fuse was inserted at C (Fig. 4).

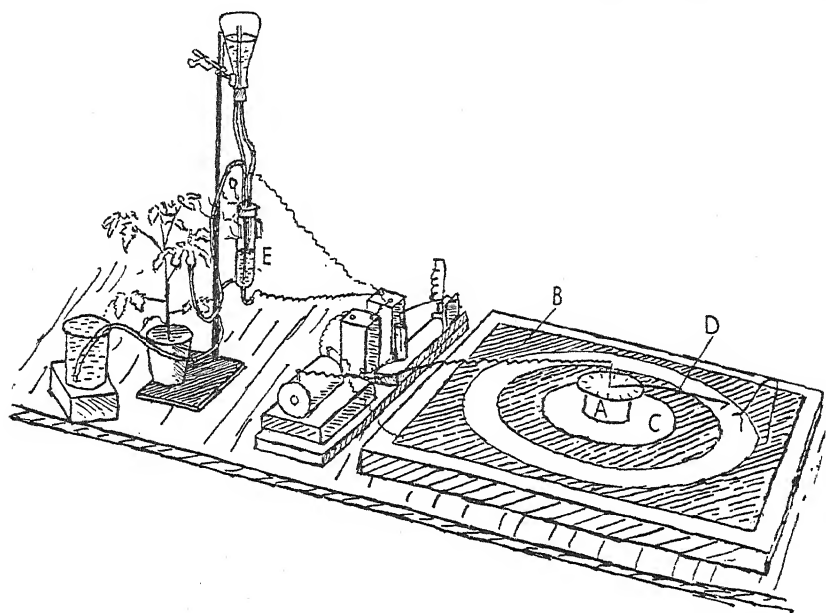


FIG. 2. Knight's porometer and the recording apparatus. (For description see text.)

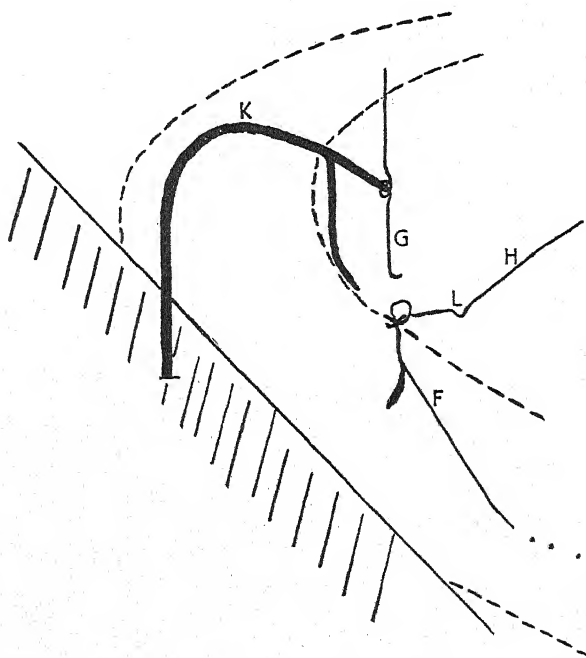


FIG. 3. Trip device for obtaining a twenty-four hour record with a twelve-hour clock. F and G are the wire trailers; H is the hour-hand arm of the clock.

Using Knight's gelatine technique, a leaf of a seedling tomato plant was attached to the porometer, and the bubbles were recorded for twenty-four hours. Fig. 5 shows a graph of the number of bubbles passing in

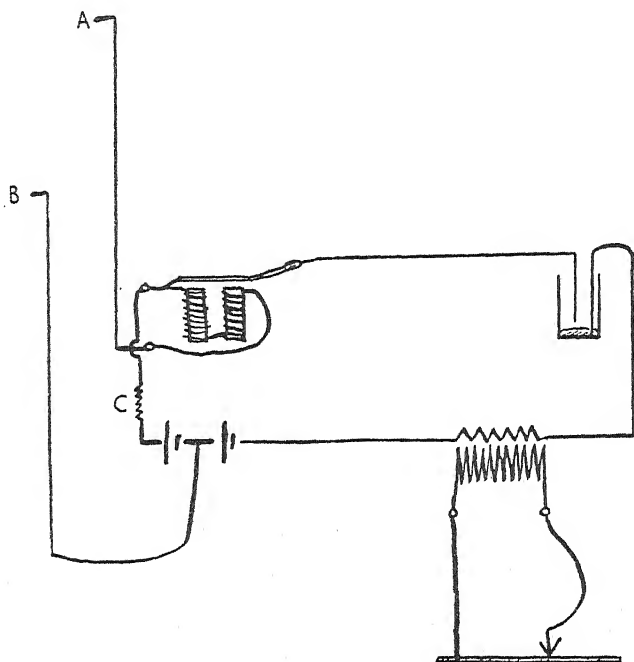


FIG. 4. Circuit diagram. A and B are the leads to the porometer; C is a fuse.

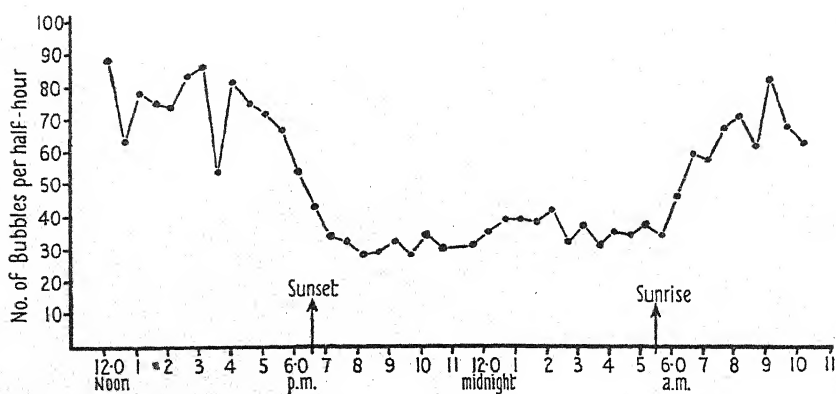


FIG. 5. Graph of bubble frequency plotted against time of day.

half-hour periods, plotted against time of day. A portion of an actual chart is shown in Fig. 6. Track A shows the typical night and B the typical day record.

At the conclusion of the experiment chloroform vapour was blown on to the leaf for ten minutes. Earlier unpublished work has shown that chloroform vapour tends to close the stomata of tomato leaflets and bubbling completely ceased shortly after this treatment, thus ruling out the possibility

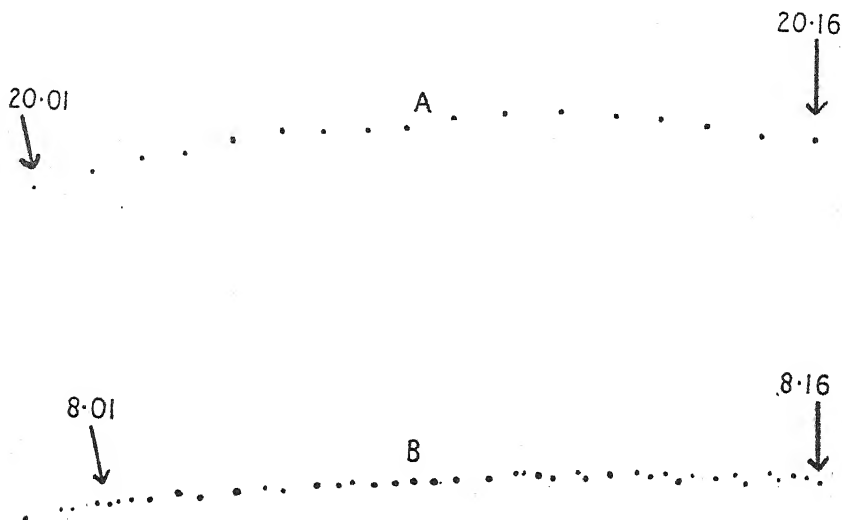


FIG. 6. Portion of a twenty-four-hour stomatal record. A = Typical night record.
B = Typical day record.

of a leaky gelatine seal. By this method correction could be made for a small constant leak at the porometer cup.

This recording device could easily be adapted for use in physiological apparatus other than Knight's porometer.

SUMMARY.

1. An improvement is described in the water reservoir used with Knight's porometer.
2. A description is given of a simple and inexpensive bubble-recording apparatus in which records over a period of twenty-four hours are obtained by perforation of a paper chart by means of sparks from an induction coil.

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The Study of the Effect of Blue-violet Rays on Photosynthesis.

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INTRODUCTION.

THE effect upon assimilation of light of different wave-lengths has been the subject of considerable investigation during the last 150 years. The work of Ursprung (13, 14) and Lubimenko (9) has shown that photosynthesis is more active in the red (760-600 $\mu\mu$) region than in the blue (480-400 $\mu\mu$) rays of sunlight. Wurmser (17, 18) concluded that green light is utilized in the photosynthetic process to about four times the extent of the red. Warburg and Negelein (15, 16), on the other hand, have shown that the efficiency of the photosynthetic system decreases with decreasing wave-lengths, and Briggs (2) also came to the same conclusion.

In the work of Briggs (2) the incident energy falling on the leaves for yellow-red, green, and blue light is not actually the same, but the rates of photosynthesis are calculated on a basis of equal incident energy. This is not satisfactory, as the rate of the photosynthetic process may not be a linear function of the incident energy. Also the intensity of radiation in the three different regions appears to be very low. With low amounts of energy it is not satisfactory to calculate the results per 500 calories per 100 sq. cm. per hour.

Burns (3) has recently studied the quantum yields in monochromatic lights, and in white light of equal intensity, and he in the main confirms the results obtained by Briggs (2). This author has also, like Briggs (2), used an artificial source of light which is very poor in the blue-violet rays (5).

The experiments of Popp (11) show that the blue-violet region is essential for the formation of carbohydrates in leaves. Similar observations

on the photosynthetic activity of algae were made by Moore, Whitly and Webster (10), and by Klugh (8), and they also find that red light is most effective for photosynthesis.

The most general conclusion is that the efficiency of the photosynthetic mechanism in green plants decreases with decreasing wave-lengths. All these experiments do little more than confirm the findings of Senebier in 1788 (12) with his simple technique of double-walled bell jars containing coloured solutions.

The work of Dastur and Samant (5) has shown that the photosynthetic process in green leaves does not proceed with the same speed in artificial light from a gas-filled electric lamp as from diffuse sunlight of the same total intensity. Their results suggest that the whole of the visible spectrum is photosynthetically effective, and a lower proportion of rays from any region of that spectrum results in a depressed rate of photosynthesis.

The rate of photosynthesis in the different lights being different, the distribution of energy in the visible regions of their spectra were determined. A study was also made of the photosynthetic activity in lights of different wave-length but of equal intensity, using as high an intensity as possible, and the results obtained were compared with those with white light of the same total intensity. Such results should indicate whether the whole visible region of the spectrum is necessary for the process, or whether the process can go on equally rapidly in any equally intense region of the spectrum.

INVESTIGATION.

The Sources of Artificial Lights Used.

In this investigation four different sources of lights were used. (1) 1,500 watt gas-filled Phillip's electric lamp; (2) 1,500 watt gas-filled Phillip's 'daylight' lamp; (3) 'Polar' incandescent oil lamp of 200 candle-power; (4) a carbon arc lamp.

The measurement of the distribution of energy in the visible part of the spectrum of each light was made by using (1) a Moll microthermopile with a D'Arsonval galvanometer, (2) by Ilford special rapid panchromatic photographic plates, and (3) by spectrum photographs of the different lights taken with an Adam Hilger's Constant Deviation Wave-length Spectrometer.

Table I gives the total intensity of the light from each source, and the intensities of the different radiations comprising the white light as measured by the thermopile.

The results of these measurements of the intensities of different rays of the visible light from different sources can be summarized as follows:

(1) The intensities of the radiations of the different constituents of white light obtained from diffuse sunlight are nearly equal. The direct sunlight is slightly more intense in the red region than diffuse sunlight.

(2) The 'daylight' bulb has a higher intensity in the red region than the direct or diffuse sunlight. The intensity of the blue-violet region stands midway between sunlight and an ordinary electric lamp.

(3) The ordinary electric light has its highest intensity in the red region, while the intensity of the blue-violet region is even less than that of the same region in the daylight bulb.

(4) The light from the incandescent oil lamp is particularly poor in blue-violet and green rays.

TABLE I.

The Intensity of Various Radiations from an Ordinary Electric Lamp, a 'Daylight' Lamp, Diffuse Sunlight, and an Incandescent Oil Lamp, all giving approximately Equal Intensities of Visible Radiation.

(Galvanometer readings in cm.)

Spectral transmission.	Colour.	Diffuse sunlight.	Ordinary electric.	'Daylight' lamp.	Oil lamp.
Total	White	5.5-7.5	8.2	8.2	8.2
6100 to red end	Red	3.4	6.45	6.2	6.8
5800 to red end	Orange-red	3.8	6.5	6.4	6.8
5600 to red end	Orange	3.8	6.7	6.3	6.0
5100 to red end	Yellow	3.9	7.2	6.85	7.4
4600-6000	Green	3.5	3.3	3.8	4.7
4200-5400	Blue	3.5	2.5	3.05	2.4
4000-5100	Blue-violet	3.2	2.35	2.95	2.2
6400 to red end } and 3800-4600 }	Violet	3.6	5.45	5.45	4.8

If the blue-violet region has any important role in the formation of carbohydrates the carbohydrate content of the leaves exposed to the daylight bulb should be greater than those of the leaves exposed either to ordinary electric light or incandescent oil lamp. The determination of the carbohydrate content of the leaves and petioles of plants exposed to these three sources of illumination was therefore undertaken. The technique of experimentation was the same as adopted by Dastur and Samant (4 and 5).

Table II gives the results of the carbohydrate contents in gm. per 100 gm. of the fresh weight of the leaves and petioles taken together.

As there is not much appreciable difference in the carbohydrate contents of the leaves exposed to an ordinary electric lamp and an incandescent oil lamp no further experiments were made with the incandescent oil lamp.

The significance of the results from an ordinary electric bulb and

daylight bulb, given in Tables II, III, and IV were statistically tested, and P was < 0.05 ($t = 2.47$, and $n = 8$); the results are therefore significant.

TABLE II.

(1) *Nicotiana tabacum* (25 Oct. 1932).

	Reducing sugar (gm.).	Sucrose as hexoses (gm.).	Starch as hexoses (gm.).	Total carbo- hydrates as hexoses (gm.).
Dark	0.001	0.008	nil	0.009
Incandescent .	nil	0.016	"	0.016
Ordinary electric	0.002	0.014	"	0.016
Daylight bulb .	0.002	0.023	"	0.025

(2) *Boehmeria scabrella* (9 Nov. 1932).

Dark	nil	nil.	0.01	0.01
Incandescent .	"	0.056	0.013	0.064
Ordinary electric	"	0.058	0.01	0.068
Daylight bulb .	"	0.063	0.013	0.076

(3) 16 Nov. 1932.

Dark	nil	0.005	0.007	0.012
Incandescent .	"	0.019	0.014	0.032
Ordinary electric	"	0.026	0.013	0.039
Daylight bulb .	"	0.035	0.016	0.051

TABLE III.

(1) *Nicotiana tabacum* (16 Feb. 1933).

	Reducing sugars (gm.).	Sucrose as hexoses (gm.).	Starch as hexoses (gm.).	Total carbo- hydrates as hexoses (gm.).
Dark	0.002	0.021	nil	0.023
Ordinary electric	nil	0.021	0.008	0.029
Daylight bulb .	0.002	0.136	0.007	0.146

(2) 24 Feb. 1933

Dark	nil	0.023	0.004	0.027
Ordinary electric	"	0.026	0.004	0.03
Daylight bulb .	0.003	0.041	0.005	0.049

(3) 4 March 1933.

Dark	nil	0.003	0.006	0.008
Ordinary electric	0.003	0.028	0.007	0.038
Daylight bulb .	0.003	0.041	0.011	0.056

The results given in Tables II, III, and IV show that the carbohydrate content of the leaves exposed to the daylight bulb is greater than the carbohydrate content of the leaves exposed to ordinary light. In some of the experiments (Table IV, Experiments 1, 2, and 3) there is no indica-

tion of photosynthetic activity, as the carbohydrate content of the leaves shows no increase over that of the leaves in the dark. As the total light energy supplied from the two sources of light is equal, the differences in the photosynthetic activity can be attributed to the differences in the energy distribution in the spectra of lights from the two sources. As the differences in the distribution of energy in the white light from the different sources lie in the blue-violet regions, the differences in the photosynthetic activity are probably due to the differences in the intensities of the blue-violet rays. In order to test this conclusion the second part of the investigation was undertaken.

TABLE IV.

(1) *Boehmeria scabrella* (20 March 1933).

	Reducing sugar (gm.).	Sucrose as hexoses (gm.).	Starch as hexoses (gm.).	Total carbo- hydrates as hexoses (gm.).
Dark	nil	0.004	0.004	0.007
Ordinary electric	"	nil	0.008	0.008
Daylight bulb .	"	0.007	0.011	0.018

(2) 30 March 1933.

Dark	0.002	0.002	0.008	0.011
Ordinary electric	nil	nil	0.011	0.011
Daylight bulb .	0.005	0.035	0.019	0.058

(3) 7 April 1933.

Dark	0.003	nil	0.015	0.018
Ordinary electric	0.002	"	0.010	0.013
Daylight bulb .	nil	0.0076	0.026	0.034

*Photosynthesis in Red Light, Blue Light, and White Light of Equal
Total Intensities.*

In view of the results described above it was considered of interest to study the photosynthetic rate in the blue-violet region and compare it with the rate of photosynthesis in equally intense red light and equally intense white light. To obtain monochromatic light it was necessary to use solutions of dyes or salts in order to get large beams of light under which plants can be exposed for a number of hours.

After several trials a solution of carmine (E. Merck) in a lithium carbonate solution of the following strength seemed to serve well the purpose of obtaining red light.

0.3 gm. of carmine. 0.5 gm. of lithium carbonate. 100 c.c. of water.

A layer of this solution 1 cm. thick allows only the red rays to pass. It was necessary then to determine the range of transmission of this solution in order to know the wave-length supplied to the plants. It was also necessary to know the percentage transmission, i.e. the maximum percentage of the light transmitted in that particular range.

First, the solution of carmine was examined in a spectrometer and the total range of transmission was found to be 6,200–7,000 Å°. The wave-length in this range was determined when the percentage transmission was at its maximum; this value may then be used in order to equalize the total intensity in any two lights. This indirect method is adopted as no single instrument correctly records the intensities of both red and blue rays. The percentage transmission was determined by means of a Hilger's Nutting Photometer used in conjunction with the Hilger Constant Deviation Wave-length Spectrometer.

The following table gives the total transmission at different wave-lengths in the spectral range transmitted by the carmine solution filter.

TABLE V.

Percentage Transmission by the Carmine Filter.

	Total range of transmission = 6200–7000 Å°.					
Wave-length	6200	6300	6400	6500	6600	6800
% transmission	4.17	7.58	17.37	33.88	43.65	46.77

From this Table it is seen that the maximum transmission is at 6,800 Å°.

To obtain blue-violet rays the following solution was used: 10 c.c. of 20 per cent. ammonia. 20 c.c. of 1.05 N copper sulphate solution. A layer of this solution 1 cm. thick has a range of transmission from 4,000–4,720 Å°. The percentage transmission at different wave-lengths in the range of light transmitted by the filter was determined as before, though Bhagvat and Dhar (1) have given these data.

TABLE VI.

Percentage Transmission of the Copper Sulphate Filter.

	Range of transmission = 4000–4720 Å°.				
Wave-length	4200	4280	4370	4470	4590
% transmission	23.9	19.9	9.9	2.8	0.70

The maximum transmission is 23.9 per cent. at 4,200 Å°.

Knowing the maximum total transmissions of the two filters, it is possible to make the transmissions equal, and hence the intensity. This indirect method has to be followed, as the thermopile is more sensitive to the rays of longer wave-lengths than to the shorter wave-lengths.

The process of equalizing the total transmissions was carried out as follows. A well covered Phillip's 45 c.p. electric tungsten bulb was used as the source of light. The heat rays of longer wave-length were removed

by 7.5 cm. of water, and by 1 cm. of 15 per cent. copper sulphate solution. A 1 cm. layer of distilled water is then placed in front of the copper sulphate solution and the amount of light passing through determined by means of a microthermopile. The light transmitted by 1 cm. of the carmine solution is then determined by exchanging the distilled water for the carmine solution. From these readings the maximum amount of light transmitted by the filter can be calculated. From this the amount of light that should be transmitted if the total transmission were 23.9 per cent. (this being the percentage transmission of the blue filter), can also be calculated. The thermopile readings are next brought down to this calculated value by putting glass plates, about 1.7 mm. thick, before the red filter till the calculated value is reached.

The following points should be observed: (1) The apparent values obtained for the total transmission of the red filter by this method are untrustworthy, for these readings vary with the distance of the thermopile from the light source. As, however, the calculated value on the galvanometer scale for a total transmission of 23.9 per cent. will vary in the same proportion, the number of plates required is always the same. (2) The reduction of light intensity by the glass plates is not directly proportional to the number of plates used.

In the same way the intensity of white light is also made equal to that of the light passing through the filter by making the total transmission equal to 23.9 per cent.

The total transmission of white and red light is made equal to that of the blue-violet filter for two reasons. Firstly, the percentage transmission by the blue-violet filter being the lowest it is easier to reduce the transmissions, and hence the intensities of the other two lights, than to increase the transmission of light by the blue-violet filter. Secondly, a blue-violet filter cannot be used in the above way as the thermopile is not sufficiently sensitive to the blue-violet rays.

The experiment was repeated six times, and it was determined finally that in order to make the intensities of the red light and white light equal to the intensity of the blue-violet light, four glass plates about 1.7 mm. thick were required with the carmine filter, and six plates of the same thickness with white light. In this way the intensities of the red light, blue-violet light and sunlight are equalized.

In order to show that the carmine and the blue-violet filters allow only the red and the blue-violet regions of the spectrum lying between 6,200–7,000 \AA and 4,200–4,720 \AA respectively to be transmitted, spectrum photographs of the transmitted region were taken by means of Hilger's Constant Deviation Wave-length Spectrometer. The glass plates used to reduce the intensity do not absorb any radiation of either the red rays or the white light.

Even though the incident light intensities are made equal in the three cases by using glass plates, it is not correct to assume that the energy absorbed by the plants from the three different lights of equal intensities is equal. According to Briggs (2) the percentage of energy absorbed diminishes with the decrease in wave-length. If this is so there is justification in decreasing the intensity of the red rays and of the white light by means of glass plates, and leaving the blue-violet region as it is transmitted by the filter.

Experiments with Monochromatic Lights.

In order to make the intensities of white light equal to that of the blue light (which is the least intense of all) it was necessary to cut down its intensity, and consequently with electric lamps the low intensity of light was limiting. It was found from the results of several experiments with an electric lamp as the source of light, that the intensity of each kind of light was too feeble for the photosynthetic process. Sunlight was therefore tried as the source of light. In Bombay it is possible to work with sunlight during certain hours of the day, especially from 10 a.m. to 3 p.m., when the distribution of energy is more or less uniform. Sunlight has the advantage that a high intensity of blue-violet rays is available.

The apparatus as used with the electric light was employed in these experiments. Potted plants, previously kept in the dark, are exposed for six hours from 9 a.m. to 3 p.m. to the red light, blue light, and ordinary sunlight. The plants are surrounded with thick dark cloth to prevent extraneous light reaching them. The filters are frequently stirred. The temperature below the water-coolers is kept the same as the outside air.

In the first four experiments (Table VII) the intensity of sunlight was not the same as that of the blue or red light, though these were equal. In the rest of the experiments (Tables VIII-IX) the intensity of sunlight was made equal to that of the red and blue lights by glass plates as stated above. Only two potted plants could be exposed each day to the red and blue light, so the experiment was repeated the next day, and the carbohydrates of four plants exposed to each kind of light on the two consecutive days were analysed together.

Dastur and Samant (5) analysed the carbohydrate of the lamina only of the leaves. It is important to analyse the petioles as well, as some of the carbohydrates formed in the leaves may have been conducted away to the petioles.

The results of six sets of experiments with the leaves of *Helianthus annuus* and *Raphanus sativus* confirm the results obtained above. Even when the intensity of sunlight is reduced and made equal to that of the blue and red light, the quantity of carbohydrates formed is greater in sunlight than in the monochromatic light. In blue light there is very little

increase in the total carbohydrate value over the values of carbohydrates found in the leaves before exposure. In Experiment (2), Table VIII, there is a decrease in the total carbohydrate content as compared to the carbohydrate contents of the leaves before exposure.

Table IX gives additional results with the leaves of *Raphanus sativus*.

TABLE VII.

Experiments with Sunlight.(1) *Abutilon asiaticum* (21 and 22 Dec. 1931).

	Reducing sugars (gm.).	Sucrose as hexoses (gm.).	Starch as hexoses (gm.).	Total carbo- hydrates as hexoses (gm.).
Dark . . .	0.002	0.017	0.074	0.093
Sunlight . . .	0.004	0.124	0.608	0.735
Red light . . .	nil	nil	0.133	0.133
Blue light . . .	"	"	0.093	0.093

(2) 30 and 31 Dec. 1931.

Dark . . .	0.004	0.031	0.057	0.092
Sunlight . . .	0.004	0.141	0.473	0.617
Red light . . .	0.002	0.038	0.137	0.177
Blue light . . .	0.002	0.026	0.077	0.104

(3) *Raphanus sativus* (13 and 14 Jan. 1932).

Dark . . .	0.003	0.011	0.024	0.037
Sunlight . . .	0.010	0.075	0.068	0.142
Red light . . .	0.005	0.016	0.036	0.057
Blue light . . .	0.006	0.013	0.029	0.049

(4) 5 and 6 Feb. 1932.

Dark . . .	0.003	0.009	0.011	0.023
Sunlight . . .	0.009	0.067	0.053	0.122
Red light . . .	0.007	0.022	0.029	0.058
Blue light . . .	0.006	0.015	0.021	0.042

In comparing the results with white and red light (Tables VIII and IX), $P = 0.01$ approximately ($t = 4.484$, and $n = 5$); therefore the differences are highly significant.

Similarly, for the results with red and blue light (Tables VII and IX), $P = 0.01$ approximately ($t = 4.974$, $n = 9$); therefore the differences are highly significant.

Formation of Starch in Light from a Carbon Arc Lamp.

The results obtained in the earlier part of this investigation conclusively show the importance of blue-violet rays in assimilation. The light from a carbon arc is rich in violet rays and was tested for the distribution of energy in the visible region by the methods described above.

TABLE VIII.
Experiments with Sunlight.

(1) *Helianthus annuus*, small-leaved variety (17 and 18 Feb. 1932).

	Reducing sugars (gm.).	Sucrose as hexoses (gm.).	Starch as hexoses (gm.).	Total carbo- hydrates as hexoses (gm.).
Dark . . .	nil	0.015	0.068	0.083
Sunlight . . .	0.002	0.045	0.294	0.340
Red light . . .	nil	0.017	0.116	0.134
Blue light . . .	„	0.016	0.077	0.093

(2) 26 and 27 Feb. 1932.

Dark . . .	nil	0.068	0.029	0.098
Sunlight . . .	0.0013	0.117	0.136	0.254
Red light . . .	0.0014	0.116	0.017	0.134
Blue light . . .	nil	0.042	0.017	0.059

(3) 4 and 5 March 1932.

Dark . . .	0.014	0.088	0.012	0.114
Sunlight . . .	0.006	0.131	0.146	0.282
Red light . . .	0.002	0.127	0.018	0.146
Blue light . . .	0.005	0.100	0.013	0.118

(4) 11 and 12 March 1932.

Dark . . .	0.003	0.100	0.025	0.127
Sunlight . . .	0.004	0.194	0.040	0.237
Red light . . .	0.006	0.141	0.026	0.174
Blue light . . .	0.004	0.119	0.033	0.156

TABLE IX.

(1) *Raphanus sativus* (18 and 19 March 1932).

	Reducing sugar (gm.).	Sucrose as hexoses (gm.).	Starch as hexoses (gm.).	Total carbo- hydrates as hexoses (gm.).
Dark . . .	0.009	0.312	0.012	0.052
Sunlight . . .	0.031	0.13	0.016	0.176
Red light . . .	0.012	0.094	0.008	0.114
Blue light . . .	0.023	0.06	0.01	0.092

(2) 25 and 26 March 1932.

Dark . . .	0.012	0.018	0.033	0.063
Sunlight . . .	0.013	0.100	0.066	0.179
Red light . . .	0.013	0.096	0.022	0.131
Blue light . . .	0.010	0.033	0.02	0.063

Spectrum photographs of the light from the carbon arc lamp were taken, and they clearly showed the greater intensity of the blue-violet region of the spectrum compared with that of the blue-violet region of the spectrum of the daylight bulb.

As the sufficiently intense beam of light received from the arc lamp is too small to illuminate even one potted plant, it was not possible to determine the carbohydrate contents of the leaves and petioles after exposure to the carbon arc.

The leaves of potted plants were therefore exposed to the light from (1) a daylight bulb, and (2) an arc lamp. The same plants were used. They were first kept in the dark for from 36 to 48 hours to free the leaves from starch. In the case of the arc lamp only one leaf could be illuminated. The leaves selected for the test were approximately of the same age. After six hours exposure the leaf from each experiment was tested for starch.

The experiments were repeated several times, and in all cases there was formation of starch in the leaves exposed to the carbon arc lamp, while no trace of starch was visible in the leaves exposed to the daylight bulb.

The distinct formation of starch is a clear proof of the greater photosynthetic activity of the leaves exposed to light from a carbon arc lamp which is richer in the blue-violet rays than the daylight bulb. The importance of the blue-violet region of the spectrum of white light is again brought out by these experiments, though the evidence obtained is of a qualitative nature only.

DISCUSSION.

The results of the experiments show an increased production of carbohydrates in leaves of plants exposed to a gas-filled electric lamp of 'daylight' type as compared with leaves exposed to an ordinary gas-filled electric lamp. It is also shown by different methods of measurements that the daylight bulb is richer in the blue-violet rays than the ordinary lamp. In view of the results obtained by Dastur and Samant (5) and the results obtained here, it can be concluded that the blue-violet region of the spectrum plays an important part in assimilation. Sunlight is richer in the blue-violet rays than the lamps and the photosynthetic activity of the leaves, as the results of Dastur and Samant (5) show, is also greater in sunlight than in the latter. Similarly, the greater production of carbohydrates in leaves exposed to the 'daylight' bulb lamp may be also attributed to greater intensity of the blue-violet rays as compared to the intensity of the same region in the ordinary lamp.

The results obtained with sunlight and with the red and blue-violet rays of sunlight, all of equal intensity, show that the formation of carbohydrates is greatest in leaves exposed to sunlight; in the red rays it is intermediate, and in the leaves exposed to blue light it is least. So in monochromatic red and blue lights of equal intensity the photosynthetic activity is greater in the red light than in the blue-violet.

These results with red and blue light considered alone are in agreement with those of Warburg and Negelein (16) and of Briggs (2). But when the results with white light of the same intensity as the red and blue-violet lights are examined, it is seen that the formation of carbohydrates is greatest in the white light.

As the results stand, there is a clear indication that even in white light the relative amount of radiations of different wave-length is of great importance in assimilation. The results obtained with the artificial lights of equal total energy, but from different sources, show this clearly. There were marked differences in the carbohydrate content of the leaves exposed to an ordinary gas-filled lamp and to a 'daylight' bulb giving the same total intensity but with a difference in the distribution of energy in its spectrum.

If the photosynthetic process takes place in more than one photo-chemical stage it is probable that for one stage a particular wave-length of light is more efficient than for the other.

In considering the mechanism and energetics of photosynthesis, it is necessary to take into consideration the distribution of energy even in the spectrum of *white* light as well as the total energy supplied. The spectral composition and distribution of energy in the visible part of sunlight seem particularly suited, at least in the middle part of the day, to the whole process, while the lights derived from artificial sources are deficient in the blue-violet region, and therefore the process does not proceed as fast.

The few qualitative results obtained with the carbon-arc lamp confirm the above conclusion, and it is hoped to obtain quantitative results with some more delicate method of carbohydrate determination by means of which the carbohydrates of a single leaf may be accurately estimated.

SUMMARY.

A study has been made (1) of the rate of photosynthesis in artificial light from different sources, showing differences in the intensity of the different radiations, and (2) of the effect of white light and of red and blue light of equal intensity on the rate of photosynthesis.

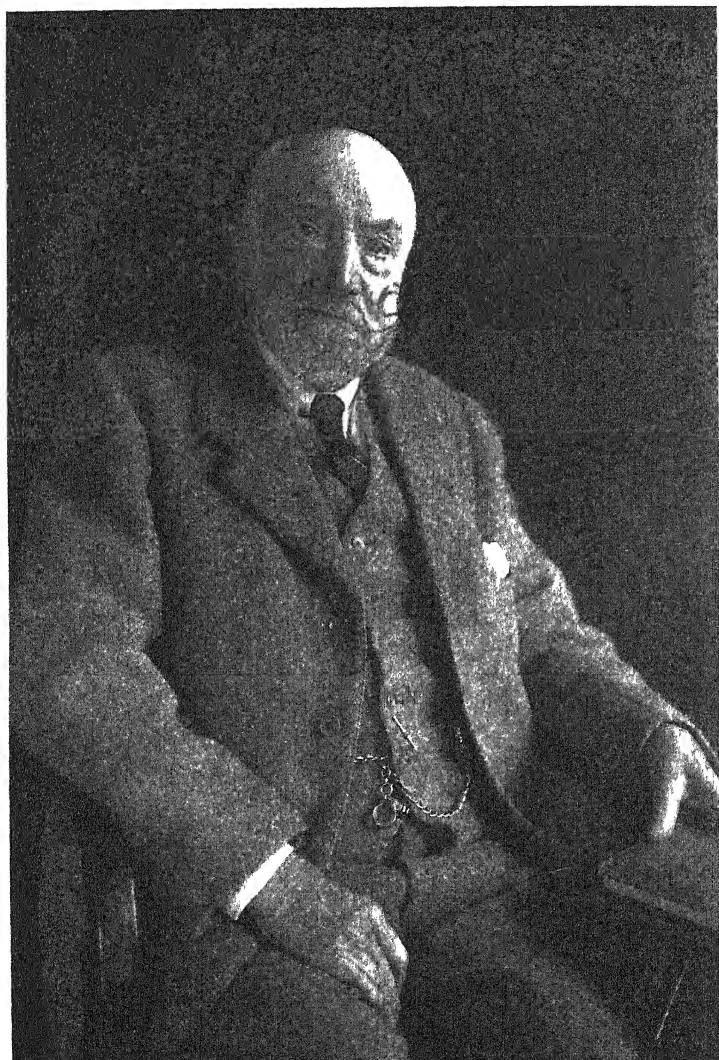
(1) The rate of photosynthesis has been studied in the light of (a) an incandescent oil lamp, (b) a 1,500 watt gas-filled lamp, (c) a 'daylight' lamp, and (d) a carbon arc lamp. The intensity of the blue-violet region in these four lamps increases in the order given. The results show that the photosynthetic rate also increases in the same order; thus the importance of the blue-violet region of the visible spectrum is demonstrated.

(2) The effect of the red rays and of the blue-violet rays of sunlight was studied. Monochromatic red light (6,200–7,000) was obtained by a specially prepared carmine solution, and the blue-violet light (4,000–4,720)

by an ammoniacal copper sulphate solution, sunlight being used as the source of light. The intensities of the red and the blue light were made equal; at the same time, the rate of photosynthesis was studied in sunlight of the same intensity. The results show that the photosynthetic activity is highest in white light, intermediate in red light, and very feeble in the blue-violet light. The results indicate that both the red region and blue-violet region are necessary for the normal photosynthetic activity, and that even with white light the assimilation depends, not only on the total energy-content, but also on the distribution of radiations of different wave-length.

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f 4

D.H. Scott 25. 9. 33

DUKINFIELD HENRY SCOTT,
1854-1934.

SUFFICIENT time has now elapsed since the death of Dr. D. H. Scott for his contemporaries to realize the nature of the gap occasioned by his withdrawal from among us. As with other great men whose intellectual distinction in combination with wide human interests and sympathy have made them natural leaders, so in the present case there can be no real successor. Nevertheless the influence which he exerted through the quality of his character will continue to animate those who knew him, whilst his almost faultless achievement in his own field of palaeobotany will remain, giving substance to its texture, and what is of greater significance, direction and ideals.

ENTRY INTO BOTANY.

Scott's entry into botany and eventual specialization in palaeobotany deserve attention as they illustrate the way in which some things come about in a haphazard world, almost as though predestined. After graduating in a non-scientific branch at Oxford he was put to a course of (railway) engineering study, influenced we may suppose by his father Sir Gilbert Scott, the well-known architect of a former generation. After three years, having become independent at his father's death in 1878, he was able to reconsider the position, and decided to take up botany as a career. It was through the influence of his mother that at the age of ten he had begun to study and identify British plants, and to develop that love of wild nature from which in later life he derived so much pleasure when he went to reside at Oakley, near Basingstoke. Somewhat later the 'Micrographic Dictionary' of Griffith and Henfrey fell into his hands, and he always declared that this was the book which definitely brought him into botany. It introduced him to the works of Hofmeister, von Mohl, and Naegeli, which were available in the Ray Society's translations, and further to the collecting and study of freshwater algae. In his own words¹—'. . . Freshwater algae I eagerly gathered. They could be examined fresh and alive. Without any troublesome preparation one learnt with the greatest ease what cells were, and how most of the algae were cellular, but others not divided into cells at all. One could watch the

¹ Quoted from his notes of an unpublished lecture entitled 'Reminiscences of a Victorian Botanist', for the use of which I am indebted to his daughter Mrs. Constance Langdon-Davies.

conjugation of *Spirogyra*, the simplest case of sexual reproduction, and could see the plant "at the moment of becoming an animal" (Unger's phrase), i.e. the cell of a *Cladophora* giving rise to a multitude of actively swarming zoospores which an uninitiated person would take for animals. I still think that the freshwater algae afford the best introduction to the elements of structural botany.'

Having decided at the age of twenty-five to pursue botany as his life's calling, Scott found his way to Kew, and was advised by Thiselton-Dyer, then Assistant-Director, to proceed to Germany, the centre of inspiration of the new botany. He accordingly went to Würzburg and studied under Sachs in 1880, and later received the Ph.D. degree; the thesis submitted, his first original investigation, dealing with the development of articulated laticiferous vessels.¹ The deepest impression made on Scott's mind by this period under such a master as Sachs, was the all importance of research; to this he remained faithful throughout his life, both in his own practice and in his advice to such as came under his influence.

Returning to England (1882) Scott accepted, though not without diffidence, the Assistantship at University College, London, at the invitation of my father. This diffidence was the natural modesty of a young man, fresh from his first piece of research, called on to give the 'good tidings' to classes of students. It is recorded that he consulted his supporter Vines (at Cambridge) as to how best he should present himself to my father at Kew, on his return from Germany. 'That is perfectly simple' said Vines, 'you have merely to go up to his room at the Herbarium and say, "Please Sir, I am the young man without encumbrances".'

At University College Scott took charge of the practical laboratory classes (1882), which the following year were extended for advanced students. In 1884 he also gave a course of lectures to the latter class, thus laying the foundation of the advanced teaching which was elaborated a few years later. These classes were open equally both to men and women students, without any intervention on Scott's part—as has been implied in some of the obituary notices that have appeared. At that time all classes in the College, except those in the Faculty of Medicine, had already been opened to women students by the Council, and as these botany classes fell technically under the Faculty of Science, the entry of women was automatic.

CONNEXION WITH KEW.

In 1885 Scott transferred to the Royal College of Science (in succession to Bower) as Assistant Professor of Botany under Huxley. Shortly afterwards he obtained the sanction of Thiselton-Dyer (who had now

¹ Scott has described his experiences at Würzburg in *New Phytol.*, vol. xxiv. 9 (1925).

become Director) to bring his advanced class to work in the Jodrell Laboratory at Kew on certain days of the week. Among the members of this class was Miss H. V. Klaassen ('Rina'), whom Scott married in 1887.

The connexion with the Jodrell also paved the way to Scotts' acceptance of the position of Hon. Keeper of the Laboratory to which he was invited by Thiselton-Dyer in 1892—a position he retained for fourteen years. Here, to the lasting advantage of Botany, Scott became the inspiring centre of research for the younger generation. The following sketch of Scott at the Jodrell is from the pen of one of the botanists who worked under his guidance at this time, and to whom I am greatly beholden for permission to include it here.

'Looking back on that time I still feel that as Honorary Keeper of the Jodrell Laboratory Scott was in a perfect position for himself and for British Botany. To a youngster, just graduated, this environment was a wonderful experience, more fully appreciated in after years. The influence of Scott carrying on his own work in his private room was in the atmosphere of the place all the time. Every day he walked through the Gardens from Richmond, and we heard him arrive. After a short interval (I suppose now that he opened his letters) he almost always strolled round the Labs. and had a talk with each one of us in turn. Often he did not say much, but stood thinking about something he had been told or seen in our work. It was our ideal, I think, to have something interesting for Scott each time he came. Botanical investigation doesn't go fast enough for that, but details that would amuse the Chief were saved up. The highest point I reached was when one morning I had found groups of sporangia on some of the fern-prothalli I was growing in the Jodrell greenhouse. Gwynne-Vaughan and I had gloated over them and waited for Scott's visit. When he came and asked if there was anything interesting he was shown the specimen. It was the time when antithetic and homologous alternation of generations were almost rival religions, and we got our fill of excitement from Scott's interest. It was not till years afterwards that he told me he first thought we were playing some trick on him!

'He always shared our ordinary daily interests and difficulties as if they were parts of his own botanical investigations—as indeed they were. It was in the way he did this that his influence in training one to be an investigator was chiefly felt. There were no formal consultations about the work, and he never went over notes or the written paper, as far as I can remember. When I see more modern methods in "organization of research", I feel how good the Jodrell way was, and how much we all owed to Scott's influence and help that never sapped independence. We all adopted him for life as a Chief—even when we had another. And through the years he remained the same, and never failed us in his interest and

help in our work. Scott was a great botanist and a great investigator, and his influence in British Botany was wide. But those who had the good fortune to work under him in the Jodrell had, I think, the cream of his influence.¹

Those who worked at the Jodrell in his own field during the Scott period (1892-1906) include Boodle, Brebner, Fritsch, Gwynne-Vaughan, T. G. Hill, Lang, Maslen, Miss Ethel Sargant, Wager, and Worsdell, whilst others, like Horace Brown and Escombe pursued researches in plant physiology.

Some idea of the nature of the problems in plant anatomy which interested Scott may be gained by perusing a critical review on recent advances in the branch printed in this Journal in 1889. Nor, when later he specialized in palaeobotany did he ever forget the living plants, whereby he avoided many of the blunders of his predecessors. The same paper also played a part in the move over to palaeobotany. Old Professor W. C. Williamson who had done so much as a collector and describer of English Coal Measure fossils, was on the look out for a younger botanist to take up his work. Advised of Scott's merits (they had not previously met), and confirmed in this good opinion by hearing the paper delivered at the meeting of the British Association at Newcastle-on-Tyne, Williamson approached Scott with his proposals.

Six months later Scott visited Williamson and had a first sight of his fossil collection. Though he has stated that he 'at once became an ardent convert to the cult of fossil plants',¹ it is characteristic that two years should have elapsed before active co-operation between them became effective. It was a serious decision likely to change the current of his life, and one not to be made in any volatile spirit. Till Williamson's death in 1895 they worked together, Scott studying the specimens and discussing his conclusions with Williamson. The three joint papers belonging to this period were written by Scott himself—every word. Such was his apprenticeship in fossil botany. Throughout life Scott remained grateful to Williamson for this inspiration, as well as full of admiration for him, both as a man and as a scientific worker.²

In 1908 Scott retired from the Keepership of the Jodrell Laboratory and migrated from the Old Palace, Richmond to Oakley, near Basingstoke.

SETTLEMENT AT OAKLEY.

The house at Oakley became a Mecca for botanists. To visit there was a joy. The setting, a farm converted to a residence with taste and skill, with three beautiful barns, upkept and adapted to the many needs of

¹ Chapter on Williamson in 'Makers of British Botany', p. 259.

² *Loc. cit.*, 260.

the family—and of the villagers too. Endless gardens with character, creepered walls, a hazel grove, larch avenue, and twin yew trees in their prime under which we sat on hot afternoons. The fertility of the soil was amazing (meadow had been broken up); I recall nasturtiums with leaves like dinner-plates, and a nearby field of wheat crossed by a public footpath, where the stalks of corn joined over our heads.

Scott's study was on the ground-floor at the end of a long wing reached by a corridor glazed on its garden side. Along the corridor hung a large picture in oils of the Cathedral at Hamburg, built by his father, Sir George Gilbert Scott, and a portrait group of some of the Scott children. The study was the sanctum where the fossil slides were kept, catalogues, and working library. The microscope table stood on the left in a bow window overlooking the garden, whilst on a platform facing the door was a windowed recess containing the writing table and cases filled with fossil slides. On such wall space as was not occupied by books were portraits of botanist friends, here and there a worthy like Darwin, and the original sketch (by Allen) for the restoration of *Lyginopteris*. This last (good effort though it may have been once) used to provoke our gibes, as it always seemed to lag behind current discovery. On the mantle shelf were appliances for smoking.

Though a sanctum, the study was frequented at proper times by all, and we generally sat there after dinner. It was more the general sense of propriety of the family than any definitely expressed wishes of the owner which determined whether the study should be invaded, or the Master left in peace to wrestle with his problems. Before retiring at night Scott used to post up his diary with such incidents of the day as were deemed worthy of record.

COUNTRY PURSUITS.

Oakley (or the Oakleys) lay in typical English country of the unspoilt type, with its share of chalk downs, woodland, cultivation, and country houses. It also has literary associations, as Steventon, where Jane Austen was born and wrote several of her novels, was within easy reach, and Selborne, made famous by Gilbert White, farther afield.

Scott had thoroughly explored this country on foot, and a walk with him was full of interest. Sometimes the objective would be one of the rarer plants in which the neighbourhood abounded (e.g. *Polygonatum*, *Iberis amara*, *Teucrium Botrys*), or some ephemeral and spectacular display such as a field of beans overwhelmed by *Orobanche*, a grove of stately mulleins, or a disturbed downland area smothered in viper's bugloss, a blue sheet visible from afar. Nor was it merely the rare or the spectacular which attracted Scott. He was a deep as well as an acute and methodical observer of the pageant of nature, and a diligent student could compile

from his letters, diaries, and occasional papers something worthy to be placed beside White's 'Selborne'.

During the last few years of his life Scott came to depend more and more on motoring as a means of getting about. By this extension of his radius he was able to visit much of the countryside previously unknown to him, and also to see more of his many friends and relations. This new distraction was a great solace to him in the severe bereavement occasioned by the death of his wife in 1929. But he did not escape the fate that is apt to overtake the ardent motorist. In those years he lost much of the physical resilience and vigour of the cross-country walker, by reason of disuse, and not through old age. None the less, these closing years had their compensations, and his faculties, human interests, and scientific enthusiasm remained unimpaired.

Beyond his special studies Scott's interests were wide. He took a strong human interest in all that went on around him—village, national and international politics; history and archaeology, especially Roman History. He was also a fisherman. He was extremely well informed, and so modest withal that it would deceive the unwary. Where he was known, as at Oakley, he was immensely respected, as was at once apparent from the bearing of those we met on country walks. This was a tribute to his simple human qualities. He was lovable.

AS A PALAEOBOTANIST.

Returning to his technical achievements, it may seem remarkable how quickly Scott took a leading position in palaeobotany. This depended really on the bent of his mind, and the fine training he had given himself as a plant anatomist.

Following Williamson's death in 1895, three new types came into his hands which exercised his faculties in quite distinct ways. These were *Cheirostrobis*, *Medullosa anglica*, and *Lepidocarpon*.

Cheirostrobis was an isolated cone from the Lower Carboniferous of Pettycur, having analogies with *Sphenophyllum* and *Calamostachys*; he made it the type of a new class. As a fructification it is the most complex of any fossil that has come to light, and its accurate description and interpretation was no mean achievement, quite apart from its theoretical aspect as a synthetic type. The mind that could grasp *Cheirostrobis* could grapple with anything.

Medullosa anglica was another valuable find, being the first British species to be recognized. Its especial value depended on its relatively simpler construction as compared with the Continental forms which belonged to a later horizon. It has proved of great theoretical interest in connecting such forms as *Heterangium* with the Cycads.

Lepidocarpon had an evolutionary interest in another direction. It

was in effect a lepidodendroid cone in which the megasporangia containing only a single functional megaspore each, became enclosed in integuments which grew up from the sporophylls, and, without dehiscence, become separated from the cone as distinct units.

These units Scott at once recognized as incipient seeds and showed that lycopods in those comparatively early days had made a definite approach to the seed habit. These seeds detached from their cones Williamson had previously detected in the matrix and had described them as a species of *Cardiocarpon*. But seed problems were not to the front in those days, and Williamson did no more than place the existence of these 'Cardiocarpons' on record. Scott's handling of *Lepidocarpon* was extremely able and made a great impression.

Meanwhile, in 1896, Scott delivered his famous course of lectures at University College, London, and published it in 1900 under the title of 'Studies in Fossil Botany'. These lectures and the book were a revelation, and aroused botanists to the significant value of fossil plant structure in relation to the evolution of the vegetable kingdom.

I remember in my student days in 1883 attending a course of nine lectures by Williamson at the Royal Institution on Fossil Plants in which the subtitle spoke of them as 'The Ancestors of the Existing Vegetation'. I also attended Scott's course thirteen years later. Broadly these two courses had in common the same subject matter, and both had regard to evolutionary aspects. Otherwise they were poles asunder; Williamson's formed the close of one epoch, whilst Scott opened up something quite different. From this time onwards Scott became the leading spirit in fossil botany.

The next important research which Scott took up was that which led to the establishment of the class 'Pteridospermae', an extensive group of fern-like plants which were in possession of seeds. This result, unlike so many others arose, not from any more or less accidentally found object from the coal mine, but from general considerations which came to a sharp focus when by searching existing collections evidence was discovered showing that the seed *Lagenostoma* more than probably belonged to *Lyginopteris*. At an early stage (1902) the matter was brought to Scott's attention as it appeared that his collaboration would be decisive in establishing a discovery so far reaching as this appeared likely to be. It is not necessary to recapitulate the circumstances here, as the narrative used by my friend Professor A. C. Seward in his admirable and detailed notice of Scott's life and work,¹ and there printed in full, gives all that is required.

To work with Scott was both an enjoyable and stimulating experience. He treated you on an equality, in spite of your inexperience, and when you lapsed into stupidity, he extricated you with tact and consideration. Thus,

¹ Obituary Notices of Fellows of the Royal Society, Dec. 1934, 216-18.

one day we were engaged in reassembling a fossil seed from three sections derived respectively from collections in Manchester, Stirling, and the British Museum. It may be explained that lapidaries in those days, when a few sections of some new or rare object had been obtained, tended to place them with different patrons, thus, literally, killing several birds with one stone. This practice of breaking up series is reprehensible as it gives enormous trouble to later investigators and must materially retard progress. I happened to be handling these three sections and was comparing the positions of the other fragments in the matrix to see how far they corroborated our idea that the three sections constituted a series from a single seed. I noticed that one of the sections showed the seed in the centre whilst the other two showed it at the edge, and pointed this out to Scott. Instead of replying that if in the case of a small object in the centre of a face of the block the lapidary should change the angle of cutting in hopes of getting a better section or of economizing the material, the object will of course fly to the edge, he said, 'These things are horribly puzzling; let's just work it out from the beginning.' He then drew an imaginary block with a small central object and made lines corresponding to the planes of the successive hypothetical sections such as would be made as the cutter changed direction, making the matter clear to the meanest understanding. 'Yes,' he added, 'now I think I understand.' To a palaeobotanist of his experience such changes in position are the merest commonplace of the method employed, and Scott had gone through all this play-acting simply to avoid a display of technical superiority which might humiliate a less experienced colleague. It would be the same in deciding whether a tissue occupying a cavity was genuinely *in situ* or merely an intrusive stigmarian appendage. These appendages are the bugbear of the beginner though the old hand recognizes them instinctively. Scott would go through the whole thing judiciously, tabulating the pros and cons, and you would be given the privilege of deciding on a matter which must have been obvious to him from the first. So you came to feel at the moment that even if your conjecture happened to have turned out wrong, you had at any rate raised a profitable discussion and that the work now rested on a surer basis in consequence!

Scott's handling and interpretation of the fossils he worked on was extraordinarily careful and sound, and he never indulged in conjectural hypotheses not justified by the material. The high standard he imposed on himself in my opinion definitely increased the reliability of work in the palaeobotanical field all round.

A striking immediate result of the *Lagenostoma*-*Lyginopteris* discovery was that it gave a lead for the interpretation of the fossil *impressions*, which, alone, in the absence of anatomical detail, were not convincing.

The first case that was made plain was *Calymmatotheca Stangeri* of

Stur, and there are numerous others to be found recorded in the text-books. The final scene in the *Lagenostoma-Lyginopteris* episode came twenty-six years later, at the Cambridge meeting of the International Botanical Congress (1930), when Dr. W. J. Jongmans exhibited numerous well-preserved specimens of impressions of *Sphenopteris Hoeninghausi* of Dutch origin with cupules and seeds attached. There was no room for further doubt. Scott was in the chair, and as the paper came to a close he silently withdrew from the meeting-room, leaving me in this 'hour of triumph' to take the 'curtain' alone. It was an exquisite gesture characteristic of the generous and scrupulous nature of the man.

With lapse of time the 'seed-fern' theory with which we had coquetted in the earlier days weakened, and the Pteridosperms drifted across to a more isolated position. The fact was, as Scott himself was the first to point out, their anatomical characters when compared with those of true contemporary Filicineae did not justify so close a linkage, in spite of their external filicinean resemblance. Moreover representatives of the group are being found too early in the geological series for it to be reasonable to derive them from the known Filicineae. The question remains open and that is all that need be said here.

Among Scott's more important contributions to fossil botany subsequent to his retirement from the Kew post (1908) may be mentioned those on *Stauropteris*, *Zygopteris Grayi*, and *Botrychioxylon* in the fern series; *Sutcliffia*, *Medullosa*, *Heterangium*, and (in conjunction with A. J. Maslen) *Trigonocarpus*, among the pteridosperms and *Mesoxylon* and *Pitys* on the cordaitean-coniferous side.

Apart from his papers on *Spencerites* and *Lepidocarpon*, Scott made no detailed investigation of the fossil lycopods though he had at one time some intention of monographing the British *Lepidodendreae* for the Palaeontographical Society. This omission merits passing comment as, architecturally, the *Lepidodendrons* being so complicated in their own particular manner, must have appealed strongly to so fine an anatomist as Scott. The possibilities in this field are now becoming apparent under the newer technique of 'transfers' and 'peel sections', as developed, e.g. by John Walton in the case of *Lepidophloios Wünschianus*.

These results would have delighted Scott as being significant of what new and appropriate methods can accomplish when properly directed. 'Transfers' have also the further advantage of placing the progress of an investigation more completely under control; for, being a method proper to the laboratory rather than the workshop, the need for the close and often rather inconvenient co-operation that formerly obtained between the investigator and the lapidary is much diminished.

SERVICES TO BOTANY.

Among Scott's great services to Botany reference must be made to his connexion with this Journal. Always a warm supporter, he was one of the active Editors from 1893 to 1912, and remained to the end of his life Chairman of the 'Annals Committee'. As Editor he was zealous in attracting what he regarded as suitable and representative papers, and if he had reason to suppose such papers were being offered elsewhere without adequate reason, the matter would be followed up promptly. His position in Botany was a definite asset to the 'Annals', and he himself published in its pages a fair proportion of his own work. Actually there are twenty-four papers in the 'Annals' from his own hand, and twelve in conjunction with others.

Though untrammelled by formal academical ties Scott was always willing to give lectures on the work that interested him to University Students, Societies, and other gatherings. He was notably generous of his time in such missions, and the quality of his lectures, which were mainly directed to the advanced student and naturalist class of audience, was of a high order. During the forty years I was at University College, beginning with the already mentioned course in 1896, he gave numerous courses of lectures—at first on the invitation of the College, later under the remodelled University of London. Other Colleges, as for instance Aberystwyth, enjoyed the same privilege. In the jargon of to-day, Oakley was a one man 'research institute', and it was by these occasional voluntary courses that Scott kept in touch with science outside and it may be clarified his own thoughts. Though, physically, he worked in isolation, there was nothing of the anchorite about Scott. Communion with others was necessary to him, and the moment a piece of work was finished he wrote out his paper and sent it for publication. His drawers were not stuffed with unfinished manuscripts. Even the receipt of proof sheets afforded him a pleasant thrill.

The services Scott gave to scientific societies were extensive. He was President (previously Botanical Secretary) of the Linnean Society, of the Royal Microscopical Society, of the SE. Union of Scientific Societies, of the Bournemouth Nat. Science Society (twice), and of the School Nature Study Union. On two occasions he presided over the Botanical Section of the British Association; he also served as Foreign Secretary of the Royal Society for a period. As a president he was felicitous both on ceremonial and less formal occasions. A remark by Miss Gulielma Lister¹ cannot be bettered, 'He brought an atmosphere of friendliness and interest to every aspect of botany when he was present at a meeting'.

¹ From Professor Seward's Obituary Notice for the Royal Society, p. 227.

A TRIP TO PARIS.

Visits abroad which included some botanical objective were much to his liking. I recall such a one to Paris in April, 1905, Scott, Mrs. Scott, my wife and I making up the party. The general idea was to examine certain specimens in which we were both interested in the various collections, including the Renault Collection at the Museum d'Histoire Naturelle, and that under Zeiller's charge at the Ecole des Mines. Our contacts with French botanists, which included Zeiller, van Tieghem, the Bertrands, Gaston Bonnier, and Grand 'Eury were very cordial, and there was no mistaking their admiration for Scott. The French have a strong tradition in fossil botany deriving from Brongniart, and were well informed as to recent English work. Perhaps the most dramatic incident of the visit was the sudden arrival of Grand 'Eury from St. Etienne with a bagful of impressions of the fronds of *Pecopteris Pluckenetii* covered with unmistakable seeds. He had just discovered them, and knowing of our visit had travelled post-haste to show them to us. Apart from the very convincing preservation, the discovery was of considerable interest, for at the time *Pecopteris* was still generally believed to be a true fern.

Apart from the ordinary relaxations of a visit to Paris, we were able to attend a meeting of the Academy of Sciences. The large meeting-room was divided into pens or pews covered in green baize where the representatives of the various subjects sat together in groups; along the wall was a shelf crowded with the busts of former members, Voltaire among them, I remember. Communications were read by the General Secretary, and the author, if present, was given an opportunity of saying a few words. Nobody appeared to pay the least attention to the proceedings, and conversation was general. The attendance was numerically good because, as it was explained to us, a certain number of attendances was obligatory to qualify members to receive the stipend attached to the position. We were also present at a lecture of Zeiller's to students at the Mining School. When we arrived the students were standing on their desks smoking cigarettes, reading the morning papers, and chattering. When Zeiller came in they subsided into their places. Later in the lecture, when some reference was made to the presence of us foreigners and the nature of our studies, they rose and acclaimed us with a charming natural courtesy.

DISTINCTIONS CONFERRED.

Scott was the recipient of numerous awards in recognition of his work from Societies and Universities, British and Foreign. He was elected into the Royal Society in 1894, even before becoming a palaeobotanist. From this Society he received a Royal Medal and also the Darwin. Though undoubtedly belonging to the select class to whom the Copley could have been

awarded, the matter never took shape. He also received the Wollaston Medal from the Geological Society and the Linnean Medal. Among distinctions from abroad he particularly valued his election as Corresponding Member of the French Academy of Sciences.

PUBLICATIONS.

The more general contributions from Scott's hand include several books and a large number of addresses, articles, and detached Lectures. He was effective as a writer from his lucid and simple style, imagination, sobriety of judgement, and philosophic outlook. His 'Studies in Fossil Botany', already referred to, is a 'classic' that more than anything else has built in palaeobotany as an integral part of morphological botany as a whole, and showed the bearing of the fossil subject-matter on the general evolutions of plants. He has also a small book in the Home University Library on 'The Evolution of Plants', and more recently, based on a course of lectures, there is his 'Extinct Plants and Problems of Evolution'. His 'Introduction to Structural Botany' (2 vols.), an elementary text-book, passed through several editions and has lately undergone revision at the hands of F. T. Brooks, thus securing for it a further period of usefulness in changing times.

Of his less bulky miscellaneous addresses and papers reference may be made to his address as President of the Botanical Section at the Liverpool meeting of the British Association (1896). This was a stirring address and woke people up. Another short general paper, 'The Old Wood and the New' (New Phytol. 1902), was also notable. From the rich material available of such smaller contributions a selection might well be reissued in collected form. Scott, when he wrote, invariably had something to say, said it well and concisely, and was always interesting.

The Scott Collection of Fossil Plant slides has found its way appropriately to the British Museum, where it is available alongside the Williamson Collection, which many years earlier, he had been instrumental in securing. Like Linnaeus's plants, the Williamson Collection would have gone abroad but for the timely intervention of Scott who advanced the purchase money to the Museum, to be repaid by convenient instalments.

With the collections are included the valuable index fully cross-referenced (largely the work of his wife) together with his own notes on the slides. The diary kept for a period of over fifty years is also at the Museum.

HIS HELPERS.

Among those who acted as assistants to Scott during the Kew period were L. A. Boodle, G. Brebner, and W. C. Worsdell. The artist G. T. Gwilliam prepared microscopical drawings over a long series of years,

whilst his fossil preparations (slides) came from the hands of James Lomax, and, to a lesser degree from W. Hemingway. These men by their scientific zeal as collectors and their good technique in section cutting have played an essential part in the advance of palaeo-botany during the last forty or fifty years.

It is never an easy task to represent on paper the nature of any man, and the greater he is the more difficult it becomes. The position of influence reached by Scott depended partly on his distinction as an investigator, partly on the human qualities which formed its background. He had industry and concentration on his objectives. He was modest and had no ambition to be clever or versatile. He knew his limitations and avoided frittering himself away in fields he had insufficiently studied. His mind was wonderfully open and his decisions were generally right. If, for example, what are called the major decisions of his life are considered, they hang logically together and created precisely the conditions of a happy and productive life. He was simple, direct, sympathetic, and easy of approach, and his memory will be cherished by all who knew him. He was invariably considerate and a most true friend. It would be difficult to find another in whom the qualities of head and heart worked together in a more perfect equilibrium.

F. W. OLIVER.

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NOTES.

A CASE OF REVERSED POLARITY IN THE EMBRYO-SAC.—A characteristic of the normal embryo-sac in the flowering plants is its marked polarity. The egg-apparatus lies at the micropylar end of the embryo-sac, and the antipodal cells at the chalazal end. Exceptions to this rule, that is, cases of reversed polarity in the embryo-sac, are very rare, and only four cases have been enumerated by Schnarf¹ in his recent book. In 1901, Lotsy² described an embryo-sac in *Rhopalocnemis phalloides* (Balanophoraceae), in which an antipodal cell lay at the micropylar end and the egg-apparatus was laterally situated. In 1915, Täckholm³ observed many irregularities in the construction of the embryo-sac in *Fuchsia Marinka* (Onagraceae), and in one case observed an embryo-sac with the egg-apparatus lying at the chalazal end. In *Lindelofia longiflora* (Boraginaceae), according to Svensson,⁴ the egg-apparatus is sometimes widely separated from the micropylar end of the embryo-sac, and in one instance lay in the chalazal region. The antipodals were not observed in this case, and perhaps had disappeared early. The largest number of such abnormal embryo-sacs has been seen by Pace⁵ in *Atamosco* (= *Zephyranthes*) *texana*, though the egg-apparatus in these cases was not situated exactly at the chalazal end. Out of 300 ovaries examined, she found in 56 ovaries 205 embryo-sacs with the antipodals in the micropylar end and the egg-apparatus on one side. Besides these four cases mentioned by Schnarf, only one more probable case of reversed polarity in the embryo-sac has been recorded recently in the sugar-cane by Dutt and Subba Rao.⁶ They found an embryo-sac with three nuclei (believed to be the antipodal nuclei) in the micropylar region, and one cell (believed to be the egg, although in the figure illustrating it the vacuole is seen on the side facing the centre of the embryo-sac) and two nuclei (believed to be the polar nuclei) in the chalazal region. Two other cells, the synergidae, were not seen, and are supposed to have degenerated.

In investigating the morphology of the family Lythraceae, we have found an instance of reversed polarity in the embryo-sac in *Woodfordia floribunda* Salisb. in material collected by us in Kumaon (Himalayas) in October 1933. This was from a single tree flowering at an unusual time, the usual flowering period of the species in this locality being February to April according to Osmaston.⁷

¹ Schnarf, K.: Embryologie der Angiospermen. Berlin, 1929.

² Lotsy, P.: *Rhopalocnemis phalloides* Jungh. A Morphological-systematical Study. Ann. jard. Buitenzorg. II ser. ii. 73-101, 1901.

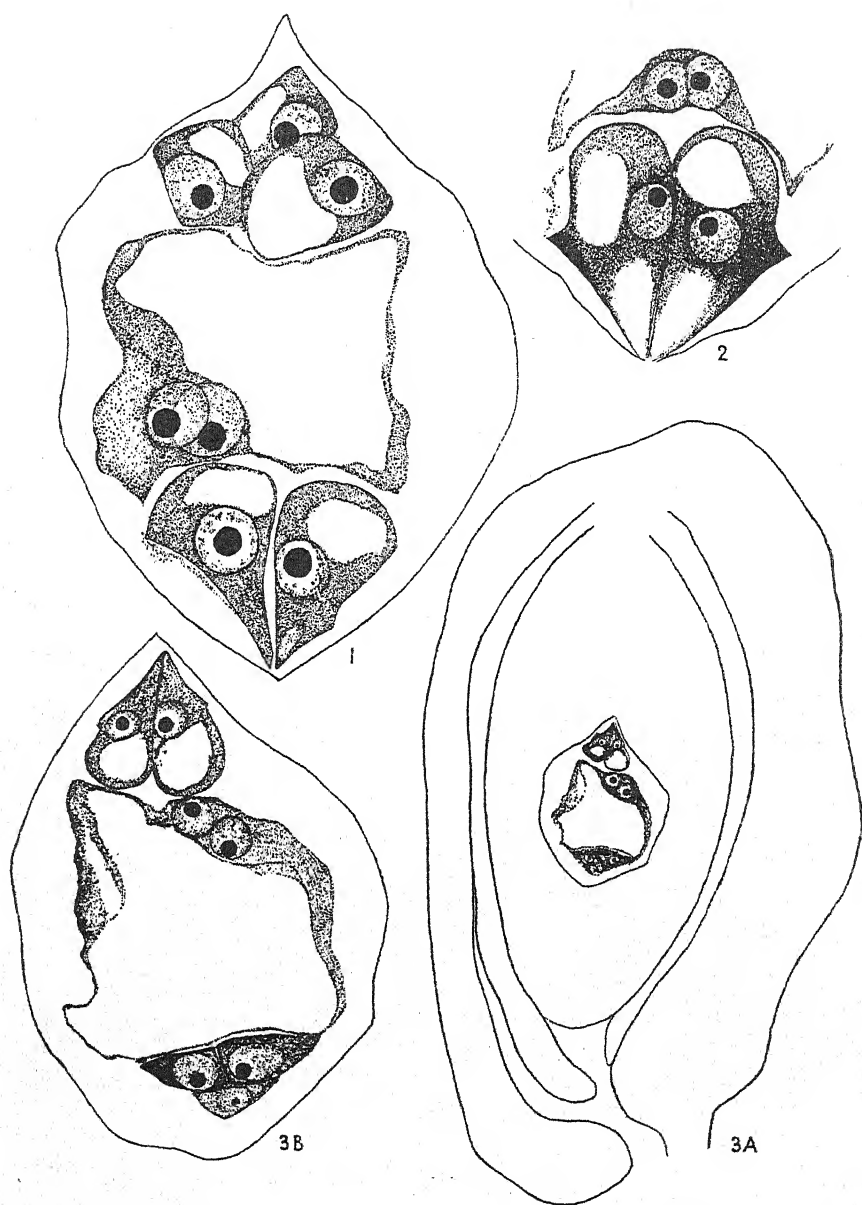
³ Täckholm, G.: Beobachtungen über die Samenentwicklung einiger Onagraceen. Svensk. bot. Tidskr., ix. 294-361, 1915.

⁴ Svensson, H. G.: Zur Embryologie der Hydrophyllaceen, Boraginaceen und Heliotropiaceen. Uppsala Univ. Arsskrift, 2, 1925.

⁵ Pace, L.: Apogamy in *Atamosco*. Bot. Gaz., lvi. 376-94, 1913.

⁶ Dutt, N. L. and Subba Rao, K. S.: Observations on the cytology of the sugar-cane. Ind. Journ. Agric. Sci., III. 37-56, 1933.

⁷ Osmaston, A. E.: A Forest Flora for Kumaon. Allahabad, 1927.



FIGS. 1-3. *Woodfordia floribunda*. Fig. 1, a normal embryo-sac showing two synergids, two polar nuclei, and three antipodals. Fig. 2, a part of the same at a later stage showing two synergids and the two polar nuclei. Fig. 3 *a*, an ovule showing an embryo-sac with reversed polarity. Fig. 3 *b*, an enlarged sketch of the embryo-sac of the same. Figs. 1, 3 *a*, and 3 *b* are reconstructions from two adjacent sections. Figs. 1, 2, and 3 *b*, $\times 1,575$; Fig. 3 *a*, $\times 520$.

The ovules of *Woodfordia floribunda* possess a many-celled primary archesporium. One of these cells cuts off a primary wall cell and develops into the megaspore-mother cell. The megaspore-mother cell gives rise to a linear tetrad of four megaspores, the chalazal one of which develops in the normal manner into an eight-nucleate embryo-sac. In form, the embryo-sac is broadly spindle-shaped (Fig. 1) and the egg has the usual form. The synergids while at an early stage develop small indentations towards the outside; these run in an oblique manner, and in different sections of the embryo-sac their distance from the micropylar tips of the synergids is found to vary. In the mature embryo-sac just before fertilization, these indentations on the synergids develop into prominent hooks (Fig. 2). Besides this peculiarity, the apices of the synergids develop prominent vacuoles. The beginning of this is seen in one of the synergids in Fig. 1, and in the fully developed condition these are seen in Fig. 2. The polar nuclei at an early stage in the history of the embryo-sac take up a position just below the egg-apparatus (Figs. 1 and 2), and unite together only about the time of fertilization. The antipodals, as in most other members of the Lythraceae (Tischler,¹; Mauritzon,²), show a tendency to degenerate early. They are, however, always organized into cells, which often develop prominent vacuoles (Fig. 1). The mature embryo-sac, a part of which is shown in Fig. 2, is always without antipodals. The central cells of the nucellus in the chalazal half are elongated, different from the rest, and connect the chalazal end of the embryo-sac with the vascular supply of the ovule ending in the chalaza. The development of the embryo agrees with that of *Lythrum Salicaria* described by Souèges.³

The ovule with a reversed polarity in the embryo-sac is shown in Fig. 3a. In Fig. 3b the embryo-sac of the same is shown on a larger scale. It agrees in all essential respects with the normal embryo-sac, except that the antipodals are situated at the micropylar pole, and the egg-apparatus and the polar nuclei at the chalazal pole. The form of the embryo-sac is slightly different, in that the micropylar end is not pointed. The antipodals have not developed vacuoles. The synergids have reached a stage corresponding to that shown in Fig. 1. Small indentations have developed on them, but vacuoles have not yet appeared in their apices.

This is the first clear case of reversed polarity observed in an eight-nucleate embryo-sac. In *Rhopalocnemis* and *Atamosco*, the egg-apparatus was only laterally displaced, and was not situated in the chalazal end of the embryo-sac. In *Lindelfia longiflora*, Svensson did not observe the antipodals. In the sugar-cane also, all the parts of the embryo-sac are not clearly described. *Fuchsia Marinka* possesses only a four-nucleate embryo-sac.

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¹ Tischler, G.: Über die Entwicklung und phylogenetische Bedeutung des Embryosackes von *Lythrum Salicaria*. Ber. d. deutsch. bot. Ges., xxxv. 233-45, 1917.

² Mauritzon, J.: Zur Embryologie einiger Lythraceen. Meddelanden från Göteborgs Botaniska Trädgård, ix. 1-21, 1934.

³ Souèges, R.: Embryogénie des Lythracées. Développement de l'embryon chez le *Lythrum Salicaria*. Compt. Rend. ac. Paris, clxxx. 949-50, 1925.

A NOTE ON THE CONDUCTION OF WATER IN FIMBRIARIA BLEU-MEANA.—The presence of numerous scales on the ventral surface of the thalli of those Hepaticae which grow in fairly exposed situations and are capable of resisting drought, was pointed out by Cavers,¹ and the extension of these scales often to the margins of the thallus suggested to the writer that their function may be associated with the conduction of water externally, not only to the prostrate or inclined thallus, but also to the rhizoidal ridge of the carpocephalum.

The following simple experiment proved this to be the case. Thalli, bearing carpocephala in all stages of development, were arranged on loose pads of cotton-wool in a Petri dish containing a dilute solution of eosin, the arrangement being such that some thalli were placed horizontally on the cotton-wool, while others were inclined at angles of 25° and 45° , so that their apices rested against the edges of the Petri dish while their distal ends only were in contact with the eosin solution, as shown in the diagram (Fig. 1). At the end of one hour the plants were examined both with the naked eye and with the aid of the binocular and reading microscopes. The function of the scales was then clearly demonstrated, for the eosin solution was easily visible in their axils, giving the whole mass of scales and rhizoids a red colour; while near the apex of the thallus where the rhizoids were few, the eosin solution could be seen in the axils of the scales only, the lateral wings which were not provided with scales being quite free from stain (Fig. 2).

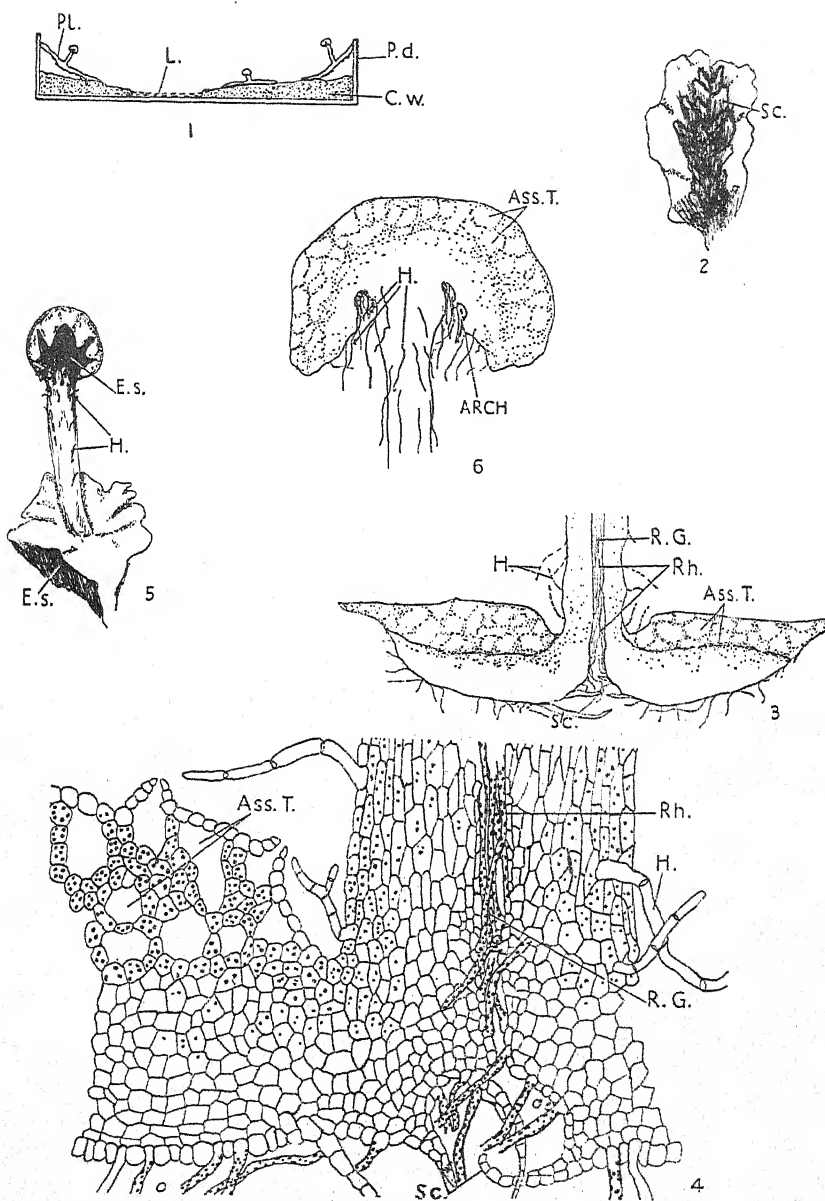
Further experiments with inclined thalli showed that the conduction of the eosin is exceedingly rapid; for as soon as the base of the thallus came into contact with the cotton-wool saturated with eosin, the spread of colour from scale to scale could be watched, and within a few minutes eosin could be seen at the apex of the thallus—a distance of from 1.5 to 2.5 cms. It therefore seems clear that a rapid rise of capillary films of liquid, passing up the narrow channels between the scales and the ventral surface of the thallus, is the major cause of this speedy conduction.

From the observations made it is evident that the scales function in at least two ways, viz. under conditions of drought they serve to retain moisture held firmly in thin films in the narrow interstices between themselves and the thallus, and under conditions of greater moisture they serve to conduct water from the older regions in contact with the soil to the apex, which is often a considerable distance from the soil overlapping the dorsal surfaces of other thalli.

Some observations were also made on the carpocephalum, with the result that it was found that a very rapid external conduction of eosin also occurs here. This rise is most rapid in the region of the rhizoidal groove, for here the tuberculate rhizoids are most numerous; but some conduction of eosin solution occurs over the whole external surface of the carpocephalum (Figs. 3 and 4).

A microscopical examination of the stalk of the carpocephalum showed that it is provided with numerous long, multicellular and thin-walled hairs which occur most freely at the base of the stalk and surrounding and protruding from the head

¹ Cavers, F., (a) Contributions to the biology of the Hepaticae. I. Leeds and London, March, 1904. (b) Notes on the Yorkshire Bryophytes. III. The Naturalist, July and August, 1904. (c) On the structure and biology of *Fegatella conica*. Ann. Bot., xviii, 1904. (d) Some points on the biology of the Hepaticae. The Naturalist, May and June, 1903.



FIGS. 1-6. 1. Diagram of apparatus used. 2. Portion of the ventral surface of the thallus of *Fimbriaria bleumeana* showing location and arrangement of the scales and the investing mass of rhizoids. 3. Diagram of a section of the thallus and part of the stalk of the carpocephalum of *Fimbriaria bleumeana* showing the rhizoidal groove with rhizoids, and the multicellular hairs on the stalk of the carpocephalum. 4. Portion of the above drawn in detail to show the tuberculate rhizoids of the groove and the nature of the hairs. 5. Thallus of *Fimbriaria bleumeana* bearing stalked carpocephalum showing the distribution of hairs on the stalk and amongst the rays of the head. 6. Diagram of a section of the head of the carpocephalum showing the location of the archegonia surrounded by the multicellular hairs.

of the carpocephalum, though they also occur less freely throughout its length (Fig. 6). It was found that films of eosin solution were retained between these multicellular hairs and the outer layer of the stalk, and this retention accounted for the rise of the fluid over the whole surface, although the most rapid conduction occurred by the aid of the tuberculate rhizoids in the rhizoidal groove.

So effective was this rise of solution over the external surface of the stalk of the carpocephalum, that accumulated drops of the coloured liquid could be seen clearly with the naked eye at the top of the stalk amongst the rays and perianth of the head. In fact, all tissues on the ventral surface of the head appeared to be bathed in it (Fig. 5).

An attempt was then made to determine how far this liquid conducted externally up the carpocephalum penetrated into its internal tissues. Thalli were treated as described above, and sections of the head of the carpocephalum were cut both transversely and longitudinally at the end of periods of four, six, and twenty-four hours respectively after the thalli had been placed on the saturated cotton-wool pads. Transverse sections showed that penetration of the eosin is most rapid into the rays of the head, but in no case was any colour evident for a depth of more than two or three layers of cells, even after twenty-four hours. Penetration of the eosin into the stalk of the carpocephalum is also very slow, and apart from a coloration of the outer two layers of cells of the stalk, eosin was not found to occur in any internal tissue. It was recognized that the absence of eosin from the internal tissues did not necessarily imply that water had failed to penetrate so far, and an attempt was made to investigate this point, and to examine the power of penetration of ordinary mineral nutrients conducted over the external surface, by placing the thalli at similar angles on pads of cotton-wool soaked in a 0.1 per cent. solution of potassium nitrate. When, however, sections of the heads of these carpocephala were placed in drops of a solution of diphenylamine in concentrated sulphuric acid, the deep blue colour given by the nitrate spread so rapidly throughout the small section that it was impossible to locate its origin. It was difficult, therefore, to settle the question as to how far the liquid travelling up over the external surface penetrated into the internal tissues, but some light was thrown on the mechanism of supply to the archegonia by sections cut longitudinally through the head after varying periods of subjection to eosin solution, when all rhizoids and hairs were seen to be deeply coloured, while little coloration, if any, was visible within the cells. Further, in all cases it was found that in the archegonia the neck cells and the egg were stained with eosin after four hours, while the base of the venter, the stalk, and the vegetative tissues surrounding the archegonium showed no accumulation of colour. So marked was the distinction that it was obvious that the eosin solution had reached the egg via the neck canal and had not passed upwards internally through the stalk. In cases of young archegonia where mucilage was still present in the canal, this mucilage must have absorbed the eosin solution fairly rapidly, while the older archegonia where the neck cells were becoming brown and

Abbreviations: *Arch.* archegonium; *Ass. T.* assimilating tissue; *C.w.* cotton-wool; *E.s.* solution of eosin; *H.* hairs; *L.* liquid; *Pl.* plant; *P.d.* Petri dish; *R. G.* rhizoidal groove; *R/h.* rhizoids; *Sc.* scales.

the mucilage had disappeared from the narrow canal of the neck, the eosin solution would reach the egg as an unimpeded capillary column. In either case the egg was rapidly bathed in the eosin solution, which had obviously reached it externally through the neck canal, and not internally by diffusion through the surrounding tissues.

The slow rate of penetration of eosin solution into the internal tissues, and the difficulty of determining the degree and location of penetration of potassium nitrate solution in the experiments described above, rendered it difficult to decide whether conduction was entirely external, or whether any slight transport of liquids might occur internally up the stalk of the carpocephalum. An attempt was made, therefore, to eliminate external conduction from the stalk of the carpocephalum and to determine whether any internal conduction could then take place.

Portions of the thallus of *Fimbriaria bleumeana* each bearing one carpocephalum were taken and placed in melted paraffin wax for 30 secs. only, the head of the carpocephalum in each case being held above the level of the wax. The wax was allowed to solidify and the bases of the stalks of the carpocephala were cut across, care being taken not to split the encrusting layers of wax. These carpocephala were then placed on portions of cotton-wool which were saturated with a 0.1 per cent. solution of potassium nitrate, in such a position that the cut ends of the stalks were in direct contact with this liquid. Transverse sections of the stalks of the carpocephala were cut after periods varying from six, twelve, to twenty-four hours and treated with diphenylamine. In no case could the presence of nitrate be demonstrated by this means for a distance of more than 2 mm. from the cut surface, and that only after twenty-four hours. It is clear, therefore, that no rapid transport of dissolved salts occurs in the internal tissues of the stalk, whether water is so conducted or not.

To settle this latter point, similar portions of thallus, each bearing one carpocephalum, were selected, as nearly uniform in size as possible, and separated into three groups. The members of the first group were treated with wax as described for the above experiment; in the case of the second group melted vaseline was substituted for the paraffin since this penetrated more effectively than did paraffin into the rhizoidal groove, and therefore eliminated more conclusively any possibility of external conduction; the members of the third group were left untreated as controls. In each case the stalks were severed very carefully from the thalli, and the stalked carpocephala were arranged on loose pads of cotton-wool which were saturated with water. They were all left in the laboratory exposed to normal room conditions of temperature and humidity, and were examined daily. The difference in the appearance of members of the three groups became increasingly obvious, and at the end of six days the carpocephala of both of the treated groups were wilted and shrivelled, while the untreated members were fresh and turgid. In this latter case both external and internal conduction, if any, were possible, while in the case of the paraffined and vaserlined plants any possibility of external conduction was eliminated. In these two cases, any water reaching the head of the carpocephalum must have been transported internally up the stalk, and the wilting in both of these groups affords a clear proof of the inadequacy of this supply.

It is obvious, therefore, that in *F. bleumeana* the water supply to the head of the carpocephalum is conveyed almost entirely externally, and that, deprived of this means of transport, this structure is unable to survive.

It is hoped to extend observations to the developing sporophyte in this species, and to undertake an investigation of the method of conduction of water in allied genera of thalloid Hepatics.

In conclusion, all the assistance given by Dr. Florence A. Mockeridge, Head of the Department of Biology, University College of Swansea, is gratefully acknowledged.

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ON THE GYNAECIUM OF *FILIPENDULA ULMARIA* MAXIM. AND *FILIPENDULA HEXAPETALA* GILLIB. : A CORRECTION.—The conclusions arrived at in the course of some detailed observations recently carried out on Malvaceae,¹ a family in which torsion of the corolla and androecium whorls in the fully developed flower renders the determination of the radial alignment of certain structures a matter of some difficulty, led me to make a re-examination of the gynaecium of *Filipendula Ulmaria* Maxim. (*Spiraea Ulmaria* L.), since in this species a pronounced lateral twist in the separate ovaries gives rise to a similar difficulty. This torsion, as is well known, does not occur in the nearly related *F. hexapetala* Gillib. (*F. Filipendula* Voss and *S. Filipendula* L.) in which the usually more numerous ovaries are bilaterally symmetrical.

In my original investigation of these two species² I formed the view that in both each ovary is composed of two whole carpels, one outer sterile and one inner fertile, the two standing on alternate radii, and suggested that the twisting in *F. Ulmaria* might result from this particular mode of construction, while the absence of torsion in the similarly constructed ovaries in *F. hexapetala* might be due to the closer packing.

The above interpretation was, of necessity, based largely on the appearances observed in the vascular system of the gynaecium since no inference could be drawn from the alignment of the carpels with the members of the outer whorls owing to the following facts, (1) that the number of perianth members in both species is very variable;³ (2) that although the trunk cords which furnish the midrib bundles of the perianth members together with those of the superposed stamen groups eventually take up their position on equidistant radii, two neighbouring cords

¹ Not yet published.

² Both species of *Filipendula*, together with other Rosaceae, were examined at an early stage in the work on carpel polymorphism (see (*), 609, 610 and figs. 157–60).

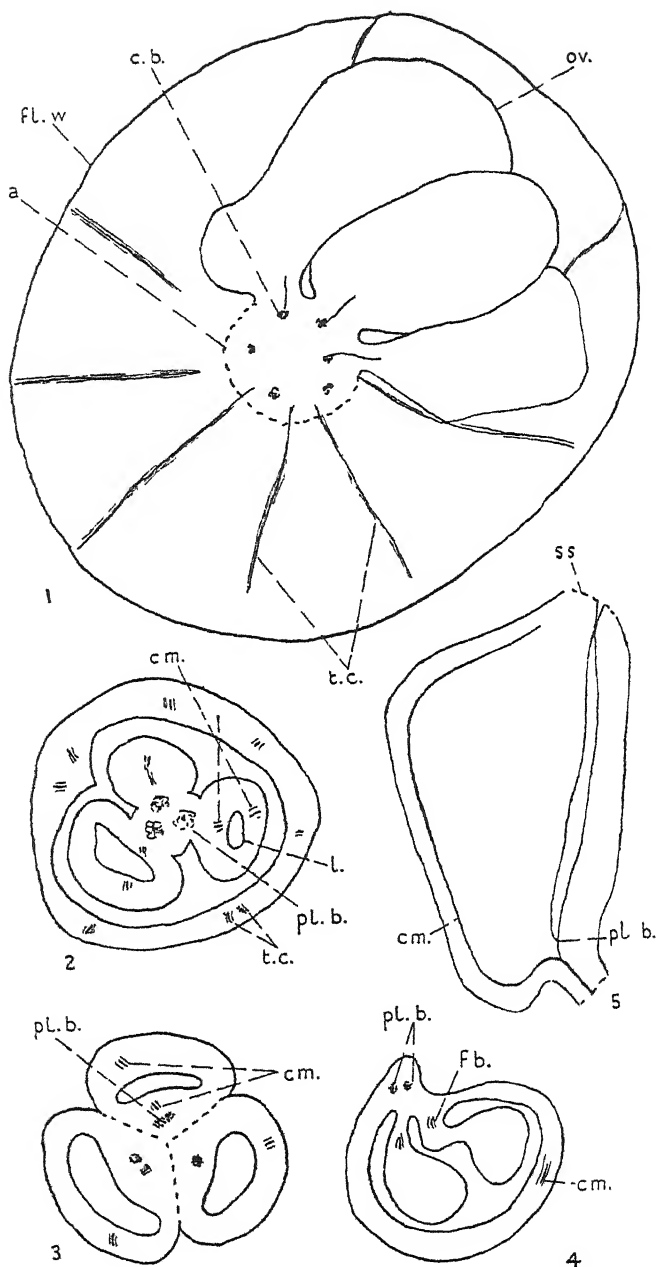
³ The only constant numerical relation appears to be that sepals and petals vary together, calyx and corolla, so far as observed, being always isomerous.

* Saunders, Edith R. : On Carpel Polymorphism. II. Ann. Bot., xli. 569–627. 1927.

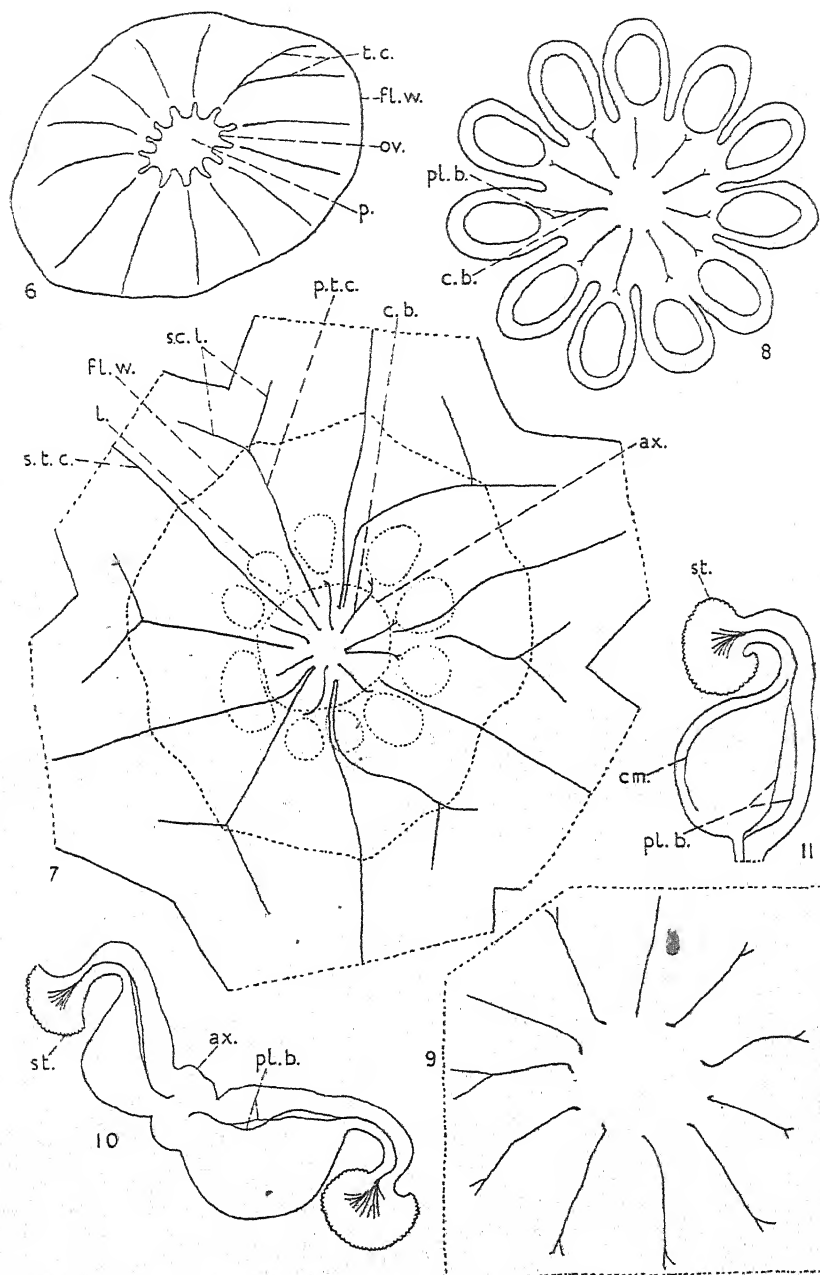
sometimes emerge from the central cylinder conjoined or, at least, from a common point; (3) that the number of ovaries in both species is also highly variable; (4) that the carpel midribs, like those of the perianth members, though finally equally spaced, sometimes arise two together at one point; (5) that the number of carpels bears no direct relation to the number of perianth members. In these circumstances the positions occupied by the ovaries afforded no clue to the number of carpels. It was concluded from the appearances observed in transverse sections that in *F. Ulmaria* the sterile and the fertile bundles of each ovary properly belong to different radii, while in *F. hexapetala* vascular elements lying between those giving rise to the sterile bundles of neighbouring ovaries appeared to be organized into the fertile bundles of these ovaries. Hence the view put forward that the ovaries in both species were bicarpellary, a construction which, as suggested, might well lead to torsion if space permitted.

In the recent re-investigation, in addition to thin cross sections, preparations were made in which the summit of the axis with the attached ovaries was rendered transparent so that the entire course of the individual bundles could thus be viewed at once. It was then evident that the inference drawn from the appearances seen in the original sections had been misinterpreted owing to the occurrence immediately before, or after, exertion of the ovary of a bend or kink in the course of the carpel bundles, the change of direction producing an effect as though midrib and placental strands had originated on different radii. The transparent preparation makes it clear, however, that in both species only a single bundle leaves the axis to supply each ovary, and that it is from this bundle that the twin placental strands take their rise. Consequently the ovaries must be regarded, not as bicarpellary, but as monocarpellary. In *F. Ulmaria* the carpel bundle, as it reaches the periphery of the axis, bends both downwards and laterally; hence in transverse sections above this level midrib and placental bundles do not appear in line. In *F. hexapetala* the midrib bundle also bends downwards, but not laterally, though this fact may easily be overlooked since, although the placental bundles are differentiated throughout the midrib bundle above, their point of departure may remain for a varying distance unligified. In this species it is at a lower level that an illusory appearance is produced, that midrib and placental strands originate side by side, since a sharp kink is often noticeable at the level at which the carpel bundle turns horizontally outwards. In passing it may be noted that in a third species, *F. kamschatica* Maxim., in which the ovaries are few and twisted as in *F. Ulmaria*, an intermediate condition was

in the centre, in slightly different stages of development. In the ovary in which the loculus has not yet appeared a portion of the midrib is seen in line with the fertile bundle left behind in the centre. In the other two ovaries the midrib which bends down under the loculus and turns sideways is seen cut twice, the portion in the outer wall of the loculus coming to lie on a radius alternating with that on which the corresponding fertile bundle stands. 3. Transverse section of the same gynaecium after the appearance of the third loculus showing the midrib of this ovary cut twice as seen in the older ovaries in 2. The corresponding fertile bundle is in process of dividing into the two placental strands. In one of the other ovaries this process is complete; in the remaining one this bundle is still single. 4. Transverse section of one of the ovaries seen in 2 and 3 showing the midrib and placental bundles 'out of line' owing to torsion. 5. Preparation of a single ovary showing the one entering bundle from which the twin placental strands are derived later. (For simplicity the ovules and the strands supplying them are omitted.) *a* axis, *c b* carpel bundle, *c m* carpel midrib, *f b* fertile bundle, *f m* flower wall, *l* loculus, *ov* ovary, *pl b* placental bundle, *s s* scar of style, *t c* tor-
 sion.



FIGS. 1-5. *Filipendula Ulmaria* Maxim. 1. Preparation of the stem apex of a K4 C4 flower with six ovaries, of which three have been removed; the remaining three are seen overlying the tissue of the flower wall. Radiating outwards to the periphery the eight trunk cords, four of which furnish the sepal midribs and antepetalous stamen bundles, the alternate four the sepal commissure laterals, petal midribs and antepetalous stamen bundles. In the centre the six ovary cords. 2. Transverse section of a K4 C4 flower with three ovaries. Surrounding the gynaecium the ring of eight trunk cords as in 1. Within the flower wall the three ovaries not yet definite.



FIGS. 6-11. *Filipendula hexapetala* Gillib. 6. Preparation from a K7 C7 flower with thirteen ovaries showing the trunk cords for sepals, petals and stamens as in 5. Above, on the right, two cords which belong to a sepal and a petal radius respectively have originated from a common point. Viewed through the pith and projecting into the central space formed as the pith comes to an end, the free ventral surface of the thirteen ovaries. 7. Preparation of a K6 C6 flower with ten ovaries showing the origin of the bundles for two neighbouring ovaries from a common point. Each bundle forks on entering the ovary to form twin placental strands. (The midrib bundle is not traceable at

observed in the differentiation of the carpel midrib, the basal as well as the distal portion being lignified, an intervening portion remaining unligified.

It follows from the above account in which it is shown that the explanation originally put forward of the twisting of the ovaries in the above species is not tenable, that the cause of this torsion is still to seek.

It only remains to emphasize the significance of the difference between the vascular scheme of the gynaeceum in the genus *Filipendula* as described above and that of related forms in which, as originally described, and as is best seen in hypogynous types, the midrib bundle of the ovaries originates from elements lying on the one set of radii and the twin fertile bundles from half the elements situated on the two neighbouring alternate radii, a system in accord with construction of the ovaries from $\frac{1}{2}$ $1\frac{1}{2}$ carpels.

The accompanying Figures were drawn by Miss D. F. M. Pertz, to whom I here tender my grateful thanks.

EDITH R. SAUNDERS.

TWO TYPES OF MODIFIED PETRI DISH.—Culture work in a heavily infected atmosphere is often unavoidable. In such conditions when the ordinary Petri dish is used a great deal of time and material is wasted by the infection of cultures and media. There are four occasions for such infection, which may occur: (1) through the edge of the closed dish, (2) when the dish is opened to pour the medium, (3) when the dish is inoculated, and (4) when the culture is examined. Of the two dishes here described, A is designed to eliminate the first three occasions and B the fourth.

Type A. The modification intended to prevent edge infection consists of a double wall at the bottom part of the dish; a 'moat' is thus formed into which the spores fall. The easiest way to improvise this is by placing the bottom of a $3\frac{1}{2}$ in. dish inside that of a 4 in. dish, and packing the space with a strip of cotton wool. The following tests provide some evidence of the efficacy of this device. All the plates were poured with malt agar and incubated for four days in a sterile chamber before being placed on a draughty bench where infection was known to be heavy. In Test I the plates were handled daily, and the position of the colonies marked, until the sixth day, when the appearance of secondary colonies brought the experi-

this level since the region connecting with the placental strands remains undifferentiated.) The position of the ovaries is indicated by the loculi which are bounded by interrupted lines, as are also axis, flower wall, and the cut edges of the sepals. (The bundle for the median lower ovary, lying partly beneath two trunk cords, is not shown). 8. Preparation of a gynaeceum of eleven ovaries. The bundles for the ovaries as in 7. 9. The vascular system of the same gynaeceum enlarged showing a kink in the course of the bundles at the point at which they turn outwards. 10. Preparation of the stem apex of another flower with two attached ovaries showing the origin of the twin placental strands from the single entering bundle. 11. Longitudinal section of an ovary showing the midrib bundle undifferentiated in the basal region and hence appearing disconnected from the placental strands. *ptc* petal trunk cord, *sc* sepal commissural laterals, *st* stigma, *stc* sepal trunk cord, *ax* axis. Other lettering as in Figs. 1-5.

ment to an end. In Test II the plates were left untouched until the sixth day when the edge colonies were counted.

Type of dish.	Number.	Position.	Edge colonies:	
			fungal.	bacterial.
I. with 'moat'	5	lid upwards	0	0
ordinary	5	" "	7	6
with 'moat'	5	lid downwards	0	0
ordinary	5	" "	1	5
II. with 'moat'	5	lid upwards	0	0
ordinary	5	" "	5	3
with 'moat'	5	lid downwards	0	0
ordinary	5	" "	0	0

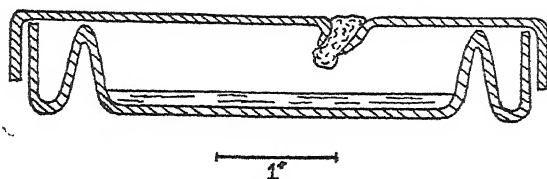


FIG. 1. Type A.

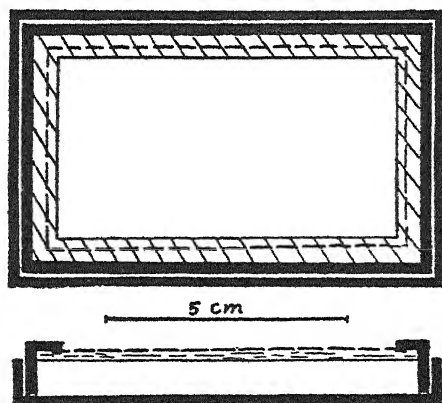


FIG. 2. Type B.

The results seem to show that with ordinary plates handling causes spores which have fallen between the edges of the inverted plates to be carried inside. The 'moat' prevents this. Further tests have shown that cotton-wool packing is unnecessary, except to keep apart the sides of the two dishes.

Infection on pouring may be eliminated by pouring the medium as hot as possible. There is of course heavy condensation which, however, soon evaporates if the plate is cooled quickly and inverted. Infection at the edge which might arise from this is prevented by the 'moat'.

Infection on inoculating can be prevented by obviating the necessity for opening the dish. A hole about 4 mm. in diameter, at the bottom of an indentation

made in the lid, will allow the entry of the inoculating wire, and can be plugged tightly with cotton wool. Fig. 1 shows a diagrammatic section through the dish with moat and perforated lid; this can be obtained through Charles Hearson & Co., Ltd. The double dish, packed with cotton wool, was the suggestion of Mr. C. S. Semmens, for whose help I am very grateful.

Type B. This (shown in Fig. 2) is a rectangular Petri dish which can be placed lid downwards on the stage of the microscope and gripped by the mechanical stage. The 'bottom' consists merely of a rectangular framework with 5 mm. ledges, over which is fitted a large cover-slip (indicated by the dotted line in the figure). The medium is poured on to this cover-slip which it seals satisfactorily to the ledge, although it can be cemented previously with glycerine jelly if required. If the medium is clear and poured thinly a $\frac{1}{8}$ in. objective can be used with ease for the examination of objects growing on its surface; the culture can thus be examined in detail without the dish being opened. As the thin layer of medium is liable to dry up rapidly it is best to keep the chamber in a larger moist Petri dish. When the mechanical stage is used the inner dish carrying the medium must be wedged in to prevent its moving and spoiling the readings.

The dish as shown in Fig. 2 is on order from Charles Hearson & Co. A fairly effective substitute can be improvised from a flat rectangular tobacco tin which has a cover-slip cemented over a hole cut in the top and in the bottom face.

This type of chamber was designed for the observation of early stages in the conjugation of Zygomycetes, for which it has been found indispensable. It has also been extremely useful for spore germinations, for spore measurements, and for isolation work.

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TIMBER AND ATTACK BY LYCTUS BEETLE.—From the analysis of uninfested timber and of the frass of the attacking larva of *Lyctus*, the Powder-post Beetle, Campbell¹ deduced that the larva depends for its nutrition rather upon the cell contents of the parenchyma cells of the sapwood of the timber than upon the cell-walls. One way, therefore, of rendering sapwood immune from the attacks of this pest would seem to be the elimination from the parenchyma of such substances as are of value to the larva as food.

Such removal of the cell contents by ordinary physical or chemical means without comminution of the wood itself is, however, not at present possible, even on a small scale, in the laboratory; the only way in which these cell contents, particularly starch, are known to diminish, is by the natural katabolism of the living cells of the parenchyma itself. During his forestry investigations, Mer² observed that if a standing tree be 'ringed' below the crown and also at ground level, the

¹ W. G. Campbell, *Biochem. Journ.*, xxiii. 1929.

² E. Mer, *Mém. Soc. Nat. d'Agric.*, cxl. 1903.

amount of starch in the parenchyma in the trunk of the tree will gradually diminish, and suggested that by some such means immunity from *Lyctus* might be secured. Later, Wilson¹ showed that if the parenchyma of felled timber be kept alive by prevention of drying, the starch in the cells will gradually disappear, and that where such disappearance has occurred *Lyctus* apparently will not attack.

The period of time required for the removal of starch by these methods is, however, a long one and extends into months, a fact that raises serious objections to their use for the prevention of attack by *Lyctus* upon timber destined for commercial use, namely, the extra labour costs in the case of the ringing of standing timber, and the likelihood of fungal attack in the case of felled timber kept moist for a long period. A study of the physiology of newly-felled timber, however, suggests a method by which these disadvantages may be overcome.

A survey of the general respiratory relationships of the wood, arising out of the writer's present work on the katabolism of newly-felled timber, discloses one set of relationships as having a more than academic importance for the *Lyctus* problem, i.e. the effects on katabolism of temperatures in the neighbourhood of the optimum.

A test series of experiments has therefore been carried out to see whether for this purpose advantage could be taken of high respiration rate. From a newly-felled, 30-year-old ash sapling, taken in March, discs 1 cm. thick and about 9 cm. in diameter were sawn and the bark peeled off. These discs were then kept in a constant temperature chamber at 33° C. and in a saturated atmosphere, one being removed periodically and tested for starch with iodine. The timber was treated as it would be under commercial conditions—after sawing, it was put straight into the chamber without the usual laboratory precautions against fungal attack, experience having shown that moulds do not readily form upon the sapwood under these conditions until death of the cells supervenes. After five days all trace of starch had disappeared. Although at the beginning the microscope showed every parenchyma cell in the wood and the rays to be packed with starch grains, yet at the end of the experiment no starch was visible.

Further experiments were carried out to find the best temperature for the process. A commercial size was chosen, the ash being sawn into planks 1 in. thick, which were kept as before in a saturated atmosphere, but at 33°, 37°, 41°, and 45° C. At 45° the starch does not disappear, the wood evidently being killed. At 41° the starch disappears in about 16 days, by which time also it has been partially lost in the other two cases. The longer period required is, of course, due to the greater thickness of the pieces and the smaller diffusion rate across the grain.

For spring-felled ash, the optimum temperature for starch diminution would thus appear to be about 41° C. This, however, is a temperature at which a modern commercial timber-drying kiln can easily be run with a saturated atmosphere for indefinite periods. Furthermore, such treatment of the timber calls for no extra handling costs, but only for the cost of running the kiln. It would seem, therefore, that this means of 'destarching' timber should be easily applicable in practice, after

¹ S. E. Wilson, *Ann. Appl. Biol.*, xx. 661, 1933.

the appropriate details of time, temperature, sap-stain precautions and so on, for different timbers and sizes, have been worked out.

Whether or not the *Lyctus*-immune timber would command the extra price is a commercial matter. For the cheaper grades it is not likely, but for the costlier woods, or for wider grades of panelling where the value is enhanced by the inclusion of the extra width afforded by the sapwood, it is probable.

The wider academic questions of the interrelation of *Lyctus* attack and chemical composition, and as to whether attack is conditioned solely by diminishing starch content or by other katabolic changes in the cell contents proceeding *pari passu*, will come under review in later communications. The purpose of this note is to record the fact that under easily-maintained conditions, living sapwood, can rapidly be rendered free from starch, and therefore, if the relationship holds good, immune from attack by *Lyctus*.

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